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MYCOLOGIA

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XLVI NOVEMBER–DECEMBER, 1954 No. 6

GROWTH OF FUNGI ON THREE SOURCES OF NITROGEN [1]

JOSEPH HACSKAYLO,[2] VIRGIL GREENE LILLY AND H. L. BARNETT

Fungi differ in their ability to utilize various nitrogen compounds for growth. Much fragmentary information on this subject is scattered throughout the literature, but comparative studies are few. Some of the early reports concerning the non-utilization of certain nitrogen compounds are of doubtful value since the need of some species for an exogenous supply of one or more vitamins was not recognized at the time the work was done. The mistaken conclusion of Fellows (**1936**) that *Ophiobolus graminis* requires complex organic nitrogen sources was due to the omission of thiamine and biotin from the medium (White, **1941**).

It has frequently been observed that species of the same genus are similar in their ability to utilize the same sources of nitrogen. With few or no exceptions, species of *Aspergillus* and *Penicillium* utilize nitrate nitrogen (Thom and Raper, **1945**; Raper and Thom, **1949**). Most species of *Fusarium* utilize nitrate nitrogen. Of the five species of *Saprolegniaceae* studied by Bhargava (**1945**) only one utilized nitrate nitrogen, and only one of the eight species of *Tricholoma* studied by Norkrans (**1950**) utilized nitrates. Lindeberg (**1944**) found one species of *Marasmius* out of thirteen to utilize nitrate nitrogen.

This study was undertaken to determine the relative amount of growth of a group of fungi when supplied with an equivalent amount

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of nitrogen in the form of nitrate, ammonium, and organic nitrogen. The influence of fumaric acid on the utilization of nitrate and ammonium nitrogen was also investigated. These experiments further served to test the generalizations of Robbins (**1937**) on the classification of organisms according to the types of nitrogen compounds utilized.

MATERIALS AND METHODS

The fungi used in this work were taken from the culture collection of the Department of Plant Pathology, Bacteriology and Entomology, West Virginia University.

The basal medium had the following composition: D-glucose, 25 gm; nitrogen source to furnish 0.425 gm N; KH_2PO_4, 2 gm; $MgSO_4 \cdot 7H_2O$, 0.5 gm; Fe^{+++}, 0.2 mg; Zn^{++}, 0.2 mg; Mn^{++}, 0.1 mg; thiamine chloride hydrochloride, 100 μg; biotin, 5 μg; double distilled water to make 1000 ml. The pH was adjusted to 5.0 before autoclaving.

The basal medium contained no chemically combined nitrogen other than the impurities in the constituents used; none of the fungi tested made a significant amount of growth in the absence of added nitrogen. Potassium nitrate (3.07 gm/1) was used as a source of nitrate nitrogen. Ammonium sulfate (2.0 gm/1) was used as a source of ammonium nitrogen. Many species make more growth on ammonium nitrogen when a four carbon dicarboxylic acid (or its salt) is present (Leonian and Lilly, **1940**). To extend our information on this subject, fumaric acid (2.0 gm/1) was added to a second series of media containing ammonium sulfate. L-Asparagine monohydrate (2.28 gm/1) was used as a source of organic nitrogen. Asparagine is widely distributed in plants and is readily utilized by many microorganisms. The many naturally occurring amino acids make the selection of a single representative source of organic nitrogen difficult.

Pyrex Erlenmeyer flasks of 250 ml capacity were used as culture vessels. The glassware was treated with sulfuric acid-dichromate cleaning solution, rinsed with tap water and finally with distilled water. Each flask received 25 ml of medium. The flasks were stoppered with cotton plugs and sterilized by autoclaving 15 minutes at 15 pounds steam pressure. They were removed from the autoclave 5 minutes after the pressure was down to avoid excessive carmelization of the glucose.

The inoculum was grown on malt extract agar in Petri dishes. Small uniform pieces of agar and mycelium about 2 ×2 mm were used to inoculate the flasks. The fungi were grown at 25 ± 1° C in a controlled temperature room which was illuminated by fluorescent lights 12 hours each day. Sufficient flasks were inoculated with each species to allow

2 to 4 harvests for each experiment. The time of harvest varied, depended upon the rate of growth. The amount of growth was determined by collecting the mycelium on a fine mesh cloth, washing, transferring the mycelium to a small aluminum cup and weighing after drying at 100° C for 24 hours. The average weights of duplicate cultures (unless noted) are reported in the tables. All of the data for a given time of incubation are taken from a single experiment. The pH of the culture filtrates was determined at the time of harvest.

EXPERIMENTAL RESULTS

The absolute rate and amount of growth for the same fungus varied somewhat in different experiments. The greatest variation occurred on the unfavorable nitrogen sources. Examples of this are given in TABLE III.

The data obtained in this investigation are too extensive to report in full. The results for each fungus for a given time of incubation, listed in TABLE I, were obtained in single experiments which should rule out variation in the basal medium, age and vigor of the inoculum, and chance variation in the external conditions.

The results presented in TABLE I indicate that all of the 25 species listed utilized organic (asparagine) and ammonium nitrogen. The Basidiomycetes, with the exception of *Polyporus distortus*, grew very slowly upon nitrate nitrogen.

The amount of growth of most species on the ammonium medium unsupplemented with fumaric acid was less than with asparagine. The value of ammonium sulfate for most of the species tested was greatly increased when fumaric acid was added to the ammonium sulfate medium Four species made more rapid or greater growth on asparagine than on ammonium sulfate supplemented with fumaric acid, while 9 species made more growth on the latter medium. The other 12 species grow equally well in asparagine and ammonium sulfate-fumarate media.

The pH values of the culture media changed during growth; the magnitude of the changes depended upon the species and the media used. The pH values of the culture filtrates from nitrate medium tended to rise to higher values, or to decrease less than those of the unsupplemented ammonium medium. The pH values of the asparagine and ammonium-fumarate media tended to lie between those of the nitrate and unsupplemented ammonium media.

Some of the fungi which utilized nitrate nitrogen well, made less growth or grew more slowly than on media containing asparagine or ammonium sulfate plus fumaric acid. A supplementary experiment

TABLE I

MILLIGRAMS OF DRY MYCELIUM PRODUCED BY 25 FUNGI ON 3 SOURCES OF NITROGEN AND THE pH VALUES OF THE CULTURE FILTRATES AT THE TIME OF HARVEST. INITIAL pH 4.7.

Ascomycetes and Fungi Imperfecti	Days	Potassium nitrate		Ammonium sulfate		Ammonium sulfate and fumaric acid		Asparagine	
		mg	pH	mg	pH	mg	pH	mg	pH
Alternaria solani	7	52	5.2	20	3.5	67	4.7	101	5.6
	12	78	6.2	24	3.6	79	5.7	103	5.6
	19	218	7.5	26	3.5	108	5.5	124	5.7
Botryotinia convoluta	7	149	5.7	53	3.4	206	4.5	164	4.9
	14	243	6.7	45	3.0	255	5.6	245	5.3
	18	235	8.6	—	—	266	6.8	234	6.4
Cephalothecium roseum	6	7	5.3	19	3.6	210	5.2	200	5.6
	11	23	5.5	19	3.7	215	5.9	207	5.6
	18	44*	5.1	26	5.4	196	5.6	185	5.3
Chaetomium globosum	4	47	5.2	22	3.5	135	3.9	106	5.7
	12	253	6.4	15	3.5	263	6.1	343	5.8
	19	254	6.0	18	3.5	193	6.1	222	5.6
Helminthosporium gramineum	5	48	5.9	51	2.9	107	4.2	129	6.2
	8	64	6.2	46	3.3	212	3.5	159	5.8
	13	178	6.6	25	2.7	301	5.1	213	5.1
Lambertella viburni	8	39	3.6	135	3.1	300	3.7	171	3.6
	12	139	3.8	196	3.1	386	4.5	378	4.4
	19	324	4.8	236	4.3	264	5.0	302	5.4
Monascus purpureus	6	46	6.0	194	2.2	278	3.8	268	4.8
	10	113	4.0	237	2.4	257	5.2	223	5.0
	17	204	4.5	212	2.6	219	5.5	233	5.6
Sclerotinia minor	10	26	3.3	30	3.2	73	2.9	78	2.8
	16	236	3.9	101	2.7	154	2.9	154	2.7
	24	327	3.5	134	2.8	113	4.0	118	2.9
Trichoderma lignorum	4	93	5.5	44	3.0	96	3.6	192	4.5
	11	205	5.3	85	3.0	184	5.4	240	5.0
	17	192	8.2	110	2.3	142	7.5	181	5.0
Verticillium albo-atrum	4	57	5.2	38	2.8	175	4.8	70	3.4
	11	170	5.1	52	2.6	226	3.4	170	3.0
	16	236	8.2	78	2.3	231	3.6	180	3.4
Xylaria mali	8	85	5.2	47	3.2	130	4.1	81	5.1
	14	263	5.7	52	3.2	307	4.8	229	5.0
	21	220	7.0	54	2.2	296	5.6	285	5.0

TABLE I.—(*Continued*)

Basidiomycetes	Days	Potassium nitrate		Ammonium sulfate		Ammonium sulfate and fumaric acid		Asparagine	
		mg	pH	mg	pH	mg	pH	mg	pH
Cyathus striatus	17	5	4.3	49	3.4	63	5.3	60	5.4
	24	4	4.0	61	3.4	142	5.2	78	4.7
	30	—	—	67	3.1	231	6.4	151	5.7
Daedalea quercina	16	2	4.2	42	2.8	36	3.9	19	4.0
	24	10	3.3	117	2.6	165	2.9	70	3.6
Exidia recisa	60	13	4.6	20	3.1	49	4.8	45	4.4
	70	25	4.3	28	2.9	126	5.8	28	4.6
Fomes subrosea	17	1	3.2	26	2.8	14	4.1	4	3.7
	25	7	3.0	63	2.9	173	3.2	22	3.5
	40	36	2.6	126	2.2	135	2.5	—	—
Ganoderma lucida	21	2	5.2	52	2.8	103	4.3	72	4.6
	40	10	5.2	77	2.7	132	6.6	171	5.8
Lenzites saepiaria	17	2	4.9	41	3.2	40	4.5	55	4.3
	25	1	4.5	71	3.1	173	4.1	291	4.0
	31	1	5.2	90	2.8	269	4.5	309	5.2
Lenzites trabea	17	—	—	46	3.0	102	3.8	45	4.1
	25	4	3.5	66	3.1	101	4.4	109	4.3
	31	13	3.9	65	2.7	89	5.8	67	6.3
Pleurotus ostreatus	10	8	4.4	33	3.7	51	4.4	29	4.5
	18	5	4.7	25	3.3	225	4.6	218	4.8
	30	25	4.3	38	2.9	339	4.2	368	4.5
Polyporus arcularius	11	2	4.5	16	4.0	44	4.4	15	4.5
	19	4	5.0	16	3.5	38	4.7	22	4.6
	31	10	4.5	51	2.8	269	4.2	291	4.3
Polyporus distortus	13	2	4.4	62	3.0	196	4.1	216	4.4
	21	4	4.9	27	3.0	134	4.0	202	3.7
	40	164	5.8	69	2.7	173	6.6	166	6.6
Polyporus gilvus	9	1	6.2	46	2.6	86	4.0	78	4.3
	15	1	4.8	60	2.9	127	5.0	139	4.3
Polyporus pubescens	6	6	4.4	22	3.1	21	4.5	10	4.2
	17	10	4.0	54	2.6	312	4.5	316	3.8
	25	10	3.9	47	2.5	257	5.2	263	5.0
Polyporus versicolor	9	14	3.5	27	3.3	115	4.1	39	3.8
	16	5	4.1	50	2.5	310	4.1	312	3.8
	24	4	4.1	45	2.5	200	4.7	212	4.5
Schizophyllum commune	13	7	3.8	24	3.3	214	4.1	256	4.7
	18	5	5.0	30	3.1	276	5.9	242	5.6
	23	6	4.5	29	3.0	213	6.3	203	5.5

* In other experiments, the yield on nitrate at 16 and 17 days was 121 and 72 mg, respectively.

was devised to determine whether fumarate would increase the initial rate of growth of some fungi or increase the total yield, when grown on nitrate medium. Two grams of fumaric acid per liter was used in these experiments. The results of typical experiments are given in TABLE II.

The results reported in TABLE II indicate that fumarate has little effect on the rate and amount of growth when nitrate nitrogen is used. A few fungi made somewhat more growth during the early days of

TABLE II

DRY WEIGHT OF MYCELIUM PRODUCED ON NITRATE MEDIUM, WITH AND WITHOUT ADDED FUMARIC ACID. INITIAL pH 4.8.

Species	Days	Without fumarate		With fumarate	
		mg	pH	mg	pH
Alternaria solani	7	36	5.7	37	5.4
	27	173	7.8	178	8.2
Cephalothecium roseum	7	41	5.7	73	6.2
	10	67	5.3	85	6.1
Chaetomium globosum	27	318	7.7	322	8.5
Helminthosporium gramineum	5	48	5.9	61	6.4
	13	178	6.6	192	7.2
Monascus purpureus	7	42	5.7	66	4.9
	10	139	5.8	126	4.6
	28	184	4.8	217	4.5
Trichoderma lignorum	4	39	5.7	40	5.5
	7	119	6.3	124	6.5
	28	234	6.9	237	7.4
Verticillium albo-atrum	4	57	5.2	109	6.9
	16	236	8.2	232	8.9

incubation in the presence of fumarate. The pH values of the culture filtrates were in some instances a little higher in the presence of fumarate.

Since the species of Basidiomycetes tested utilizd nitrate nitrogen slowly, or not at all, within the times of incubation used in the first experiments (TABLE I), it seemed desirable to determine whether fumarate would influence nitrate utilization by some of these fungi. The time of incubation was increased, while the experimental conditions remained the same as in the experiments reported in TABLE II. A portion of the data is given in TABLE III. Since the dry weights of duplicate cultures varied widely in some instances, the weights of individual cultures are given in TABLE III.

Despite the variations encountered in the experiments reported in TABLE III, it is clear that the seven species of Basidiomycetes tested are able to utilize nitrate nitrogen for growth. These species differ widely in the rate at which they grow on nitrate medium. The presence of fumarate in the nitrate medium appeared to increase the rate, or amount, of growth of some of these species, but the variation among

TABLE III

DRY WEIGHTS OF MYCELIUM PRODUCED BY DUPLICATE CULTURES OF 7 SPECIES OF BASIDIOMYCETES ON NITRATE MEDIUM, WITH AND WITHOUT ADDED FUMARIC ACID. INITIAL pH 4.8.

Species	Days	Without fumarate		With fumarate	
		mg	pH	mg	pH
Exidia recisa	70	18	4.5	28	5.8
	70	32	4.1	9	5.5
Fomes subrosea	57	64	3.0	67	2.8
	57	45	3.1	92	2.9
	74	128	2.7	219	3.3
	74	146	2.7	128	3.0
Lenzites saepiaria	62	11	3.7	19	4.8
	62	5	3.5	9	4.6
	71	22	4.0	15	4.7
Lenzites trabea	62	41	4.0	31	4.9
	62	45	3.3	32	4.6
	72	100	4.1	—	—
	72	102	3.4	53	5.3
Polyporus distortus	71	310	5.1	295	5.9
	71	373	5.5	312	5.9
Polyporus gilvus	74	28	3.3	72	4.8
	74	18	3.8	45	5.5
Polyporus pubescens	58	145	4.4	172	4.5
	58	60	4.2	187	4.6
	74	104	4.4	209	4.6
	74	90	4.4	247	4.6

duplicate cultures was too great to allow much confidence in this conclusion. This may indicate that the steps in the reduction of nitrate to ammonia are the limiting factors; or that adaptive enzyme formation is required for utilization of nitrate nitrogen by these species.

The utilization of nitrate nitrogen by some organisms is influenced by the molybdenum content of the substrate (Steinberg, **1937**; Mulder, **1948**). A nitrate medium was prepared containing 50 micrograms of sodium molybdate per liter. With the possible exception of *Cephalo-*

thecium roseum, the addition of this amount of molybdenum affected neither the rate nor amount of growth of 5 other species which utilized nitrate readily. *Pleurotus ostreatus, Cyathus striatus* and *Polyporus versicolor* did not utilize nitrate nitrogen any better when molybdenum was added to the medium.

DISCUSSION

In experiments of short duration, many of the species tested in this work utilize nitrate nitrogen slowly, or not at all. Where many species of unknown nutritional requirements are to be grown, nitrates should be considered a poor source of nitrogen. While ammonium nitrogen was utilized by all the species tested, the amount of growth of most species was restricted when ammonium sulfate was used. This inhibition of growth was presumably caused, in part at least, by the low pH values developed in this medium. The addition of fumaric acid (fumarate) maintained the pH values at higher levels and made ammonium sulfate about equivalent in value as a nitrogen source to the more expensive L-asparagine. Asparagine costs about 20 times as much as ammonium sulfate and fumaric acid.

Many of the test fungi grew slower initially on nitrate nitrogen than on asparagine or ammonium nitrogen when fumarate was present. Two of the fungi, *Lambertella viburni* and *Monascus purpureus,* made more rapid growth initially on unsupplemented ammonium nitrogen than on nitrate nitrogen. It is probable that the reduction of nitrate nitrogen and its incorporation into essential metabolites requires more time and a greater expenditure of energy than is necessary when reduced nitrogen is available. This would be especially noticeable at the beginning of an experiment when the amount of mycelium is small.

This phenomenon of slow utilization of nitrate nitrogen appears to an exaggerated degree in some of the Basidiomycetes tested. Seven species of Basidiomycetes, when incubated for about 70 days, gave evidence of utilizing nitrate nitrogen. This ability was not apparent, in most instances, by the time the maximum weight of mycelium was produced on easily assimilable sources of nitrogen. In view of these results, it appears that a latent ability to utilize nitrate nitrogen may be easily overlooked when short times of incubation are used. From the standpoint of culturing fungi in the laboratory this latent ability to utilize nitrate nitrogen may be of little value. It may have survival value under certain conditions. In view of the foregoing, it is probable that Robbins' (**1937**) classification of organisms on the basis of the nitrogen compounds they use, is less sharp than was originally supposed.

The influence of various organic acids on the utilization of ammonium nitrogen has been studied by Leonian and Lilly (**1940**), Burkholder and McVeigh (**1940**), Lilly and Leonian (**1942**), Brian *et al* (**1947**), Bernhard and Albrecht (**1947**), Lewis (**1948**), Reischer (**1951**), Morton (**1951**), and Machlis (**1953**). Two general explanations have been offered for the observed effects. The first stresses the importance of the buffer action of the compounds in maintaining the pH of the medium within favorable limits. This effect may be seen in TABLE I. In the critical pH range, a difference of a few tenths of a pH unit may profoundly affect the growth of a fungus (Lilly and Barnett, **1947**). It is doubtful if the favorable effect of certain organic acids is due solely to buffer action, for Brian *et al* (**1947**) found that the effect of malic acid on the growth of *Myrothecium verrucaria* on ammonium sulfate medium was not directly due to the effect of this acid on the pH drift of the medium.

Various investigators (Brian *et al*, **1947**; Lewis, **1948**; Reischer, **1951**) have postulated that the four carbon dicarboxylic acids which favorably influence the utilization of ammonium nitrogen do so *via* oxaloacetic and *alpha*-ketoglutaric acids generated by the Krebs cycle. These keto acids, according to recent views, are the intermediate compounds with which ammonia combines in the process of formation of aspartic and glutamic acids.

Norkrans (**1953**) studied the growth-stimulating effects of glutamic and aspartic acids, their corresponding keto acids and half amides, fumaric acid, glutathione and casein hydrolysate on seven species of *Tricholoma*. Ammonium nitrogen was used in the basal medium. In general *alpha*-ketoglutaric and oxaloacetic acids replaced glutamic and aspartic acids, and except for *Tricholoma imbricatum* were more stimulating than fumaric acid. Machlis (**1953**) has stressed the importance of pH in any interpretation of the effect of dicarboxylic acids on the growth of fungi.

In seeking an explanation of the effect of the four carbon dicarboxylic acids, another factor must be considered. From TABLES II and III it is evident that fumaric acid has little effect on the utilization of nitrate nitrogen. Nitrate nitrogen is reduced before it is incorporated into the constituents of the mycelium. The postulated pathway of nitrate nitrogen utilization of *Torula utilis* has been studied by Virtanen and Csáky (**1948**) and Virtanen *et al* (**1948**). After reduction to hydroxylamine and combination of this substance with *alpha*-keto acids, the pathways of ammonium and nitrate nitrogen are identical. Two possible explanations of the failure of fumarate to increase nitrate nitrogen utiliza-

tion are suggested. First, only the nitrate nitrogen, which has been reduced, is available for biochemical synthesis, while all of the ammonium nitrogen is potentially available. In this connection, Hopkins (**1935**) found certain nitrogen sources to be more effective for the growth of yeast when added in small doses than when the total amount was added initially. Second, at the more favorable pH of nitrate media, fungi may be able to synthesize the *alpha*-keto acids used in amino acid formation more rapidly.

The evidence presented in this paper is not sufficient to exclude either of the two hypotheses as to mode of action. Indeed, both mechanisms may operate, depending upon the species, and experimental conditions used.

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SUMMARY

The rate and amount of growth of twenty-five species of fungi were determined when they were supplied with nitrate, ammonium, and organic (asparagine) nitrogen. Nitrate nitrogen was used slowly by some of the Basidiomycetes tested and poorly, if at all, by others. From the standpoint of rapidity and amount of mycelium produced, asparagine and ammonium sulfate supplemented with fumaric acid were about equal in value and superior to either ammonium sulfate or potassium nitrate. Fumarate had little if any effect on the utilization of nitrate nitrogen.

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CONCERNING THE NUTRITION OF APODACHLYA BRACHYNEMA [1]

ROBERT H. GILPIN [2]

Within the past fifteen years there has been a growing interest in the nutritional requirements of aquatic fungi. This has been due, at least in part, to the successful isolation in pure culture of species of water molds not previously so isolated. Emerson (**1950**) has presented a summary of these isolations in tabular form. Cantino (**1950**), in his discussion of the significant contributions on the nutrition and metabolism of the water molds, provides an extensive bibliography of the literature to date on the subject. More recent studies have been published by Cantino (**1951**) on the nutritional requirements of *Pythiogeton*, by Barner and Cantino (**1952**) on the nutrition of a new species of *Blastocladiella*, by Machlis (**1953a**, **1953b**, **1953c**) on the growth and nutrition of *Allomyces* species in the subgenus *Euallomyces*, and by Machlis and Ossia (**1953a**, **1953b**) on the cultural conditions effecting maturation of meiosporangia in the subgenus *Euallomyces*.

Work reported on fungi belonging to the order Leptomitales has been limited. A brief account of the culture of certain species of *Rhipidium* and of *Araiospora* on various natural and synthetic media was presented by Minden (**1916**), and observations on some of the nutritional requirements of *Sapromyces elongatus* (Cornu) Coker (*S. Reinschii* (Schroeter) K. Fritsch) have been reported by Bishop (**1940**). Some observations on the nutritional requirements of *Leptomitus lacteus* (Roth.) Agardh. have been presented by Kolkwitz (**1903**), Trommsdorf (**1918**), Tiegs and Dorries (**1926**), and Dorries and Haase (**1930**). Schade (**1940**) and Schade and Thimann (**1940**) have presented results of apparently the most recent physiological study of any of the Leptomitales. These workers have investigated *Leptomitus lacteus* in considerable detail and *Apodachlya brachynema* (Hildb.) Pringsheim less extensively, and have compared certain of their physiological characteristics.

[1] The writer is indebted to Dr. Arthur W. Ziegler for direction of the research reported in this paper. He is grateful for advice generously given by Dr. Anne L. Pates. This work was made possible through the use of equipment provided to Dr. Ziegler under a United States Public Health Grant.

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Cantino (**1950**) has summarized and analyzed the information that has been published relating to nutrition and metabolism of the water molds and has interpreted this with respect to possible phylogenetic relationships among the water molds. He has pointed out that, on the basis of available data, the Leptomitales are physiologically different from the Saprolegniales. Cantino concludes that leptomitaceous fungi apparently have evolved along a completely independent path as indicated by their lack of ability to grow on inorganic sources of nitrogen and because they possess, in contrast with the Saprolegniales, the ability to reduce sulfates.

Many workers recognize, as pointed out by Lilly and Barnett (**1951**), that while positive results obtained in experiments to determine the utilization of a given nitrogen source are to be regarded as conclusive, negative reports should be accepted with caution. Since Schade and Thimann (**1940**) had reported negative results on utilization of inorganic nitrogen sources in the only qualitative investigation on any of the Leptomitales thus far reported, it was suggested that this phase of the nutrition of *Apodachlya brachynema* might profitably be re-examined to provide a possible confirmation of the findings of these workers. The observations reported below, taken from a more extensive study (Gilpin, **1953**) on the nutrition of *Apodachlya brachynema*, are limited to utilization by this water mold of selected carbon sources and nitrogen sources.

MATERIALS AND METHODS

The isolate of *Apodachlya brachynema* used throughout these experiments was obtained from American Type Culture Collection (*#10949*) to whom it was contributed by Dr. J. N. Couch. The fungus was subcultured and maintained in pure culture on the basal medium recommended by Lilly and Barnett (**1951**, p 427) as suitable for the laboratory culture of fungi for physiological studies.

Throughout these experiments the nitrogen sources were calculated to provide, in each case, the same amount of nitrogen that would be present in two grams of asparagine. The following nitrogen sources were used: glycine, asparagine, ammonium tartrate, ammonium chloride, ammonium sulfate, sodium nitrate, and calcium nitrate. The carbohydrates were added in direct substitution amounts for 10 gm per liter of sucrose. The carbon sources used were: corn starch, rice starch, raffinose, lactose, sucrose, maltose, glucose, galactose, and fructose.

Inocula for testing were taken from pure cultures growing on the basal semi-synthetic medium by removing with a wire loop a portion of the

hyphae from the periphery of the colony. These portions were all of the same size (6 mm^3) and each contained, within the limits of observation, essentially the same amount of mycelium. Each unit of inoculum was obtained by (1) placing a sterile wire loop (2 mm diam.) on the surface of the agar plate to include the growing tips and as much mycelium as the loop would cover, (2) pressing the loop into the agar 2 mm, and (3) removing the resulting small disk of agar containing the actively growing hyphal tips. Each plate was inoculated at three places with one of these disks in order that the results would be obtained in triplicate. Plates were incubated at a constant temperature of 15° C.

After five days of incubation on the medium containing the specific substance being tested, the growth was determined by measuring the diameter of the three colonies in each dish and ascertaining the average. Differences among the three colonies in a plate were found to be negligible. This average was recorded as the zero reading. At this time the inoculation disks were removed from the plates and incubation continued for five more days. After ten days incubation from the time of inoculation the total diameter of growth was determined again. As the final reading, the average of the three colonies in each dish was recorded. The zero reading was subtracted from the final reading to give an index of utilization and a method of comparing various substances semi-quantitatively. This method of measurement and evaluation is relatively simple to carry out.

Preliminary investigations to determine conditions most satisfactory for vegetative growth on the basal medium indicated as optimum a pH of 6.5 and a temperature of 15° C. Therefore, throughout subsequent experiments these conditions were employed as standard for culture.

RESULTS AND DISCUSSION

The results of the experiment to determine the utilization of carbon sources are provided in TABLE I.

In the absence of a carbon source, no additional growth was noted after the eighth day. The early growth on the medium to which a carbon source was not added could be due to: (1) available carbon from sucrose present in the inoculation disks which could support growth during the early days of incubation, (2) some diffusion of the sucrose from the inoculation disks into the substrate, or (3) the storage of a limited reserve of carbohydrate within the hyphae during the early period of incubation which would support some growth after removal of the disks.

TABLE I

UTILIZATION OF VARIOUS CARBOHYDRATES AS A SOURCE OF CARBON

Carbohydrate	Diam. after 5 days	Diam. after 8 days	Diam. after 10 days	Increase 5–10 days
Sucrose	1.76 cm	3.16 cm	3.96 cm	2.20 cm
Rice starch	1.80	2.66	3.96	2.16
Corn starch	1.93	2.76	4.00	2.07
Raffinose	0.72	1.67	2.50	1.78
Maltose	0.90	2.00	2.50	1.60
Glucose	1.53	2.63	2.96	1.43
Fructose	1.13	2.03	2.53	1.40
Galactose	0.90	1.57	1.90	1.00
Lactose	1.53	1.80	1.86	0.33
(No carbon)	0.23	0.50	0.50	0.27

Growth on lactose was only slightly greater than the maximum growth observed on the medium lacking a carbon source, so there remains some doubt as to whether this sugar can be utilized at all. It should be noted that, in these experiments, growth resulted on media in which either maltose or galactose was the carbon source. Schade (**1940**) had previously reported that these sugars would not support growth of *Apodachlya brachynema*.

The results of the experiment to determine the utilization of nitrogen provided by different nitrogen sources are shown in TABLE II.

Since these results were not in accord with the findings of Schade (**1940**) because it was observed that *Apodachlya brachynema* appeared capable of utilizing inorganic nitrate- and ammonium-containing compounds as a source of nitrogen, it seemed necessary to test these results further. To do this, four flasks of basal medium, from which asparagine and agar were omitted, were prepared. To one flask sodium nitrate was added to give the equivalent amount of nitrogen that would be present in the amount of asparagine included as a component of the basal semi-synthetic medium. A standard inoculation disk from the colony grow-

TABLE II

UTILIZATION OF VARIOUS NITROGEN-CONTAINING COMPOUNDS AS A SOURCE OF NITROGEN

Nitrogen source	Diam. after 5 days	Diam. after 10 days	Increase 5–10 days
Sodium nitrate	1.35 cm	3.05 cm	1.70 cm
Ammonium tartrate	1.28	2.91	1.63
Calcium nitrate	0.95	2.33	1.38
Ammonium chloride	1.31	2.43	1.12
Ammonium sulfate	1.15	2.13	0.98
Asparagine	0.38	1.25	0.87
Glycine	0.53	1.26	0.73

ing on the agar plate culture in which sodium nitrate provided the only nitrogen source was placed in this liquid medium. The same procedure was followed, also, in testing for utilization of nitrogen from calcium nitrate, ammonium chloride, and ammonium sulfate in chemically definable liquid media.

The growth of the fungus from the inoculation disks derived from the petri plate cultures utilizing a solid medium containing as the nitrogen source the same one as was provided in these liquid media, continued throughout the duration of the experiment (10 days). Examination of the mycelium in these cultures with a compound microscope at a later date revealed that oogonia and antheridia had developed normally on the growing hyphae in all of the flasks. This would seem to offer conclusive evidence that in the presence of sucrose this fungus is capable of utilizing inorganic nitrate and ammonium nitrogen for normal development.

The differences in the results obtained in this study and those reported by Schade (**1940**) might be attributed to (1) an interdependence between the carbon source employed and the nitrogen source provided, in which case certain nitrogen sources may be utilized in the presence of one carbon source but which are unavailable to this organism in the presence of another, or (2) our use of different strains of *Apodachlya brachynema* which varied in their physiological characteristics.

In view of these results it would seem that the conclusions which Cantino (**1950**) has reached regarding the importance of nitrogen utilization in indicating phylogenetic relationships among the water molds may require some revision when certain of those already investigated, and additional ones, have been studied more exhaustively.

SUMMARY

1. *Apodachlya brachynema* was found capable of growing on the following carbon sources in the decreasing order of their utilization when the nitrogen source was asparagine: rice starch, corn starch, raffinose, maltose, glucose, galactose, and lactose. Lactose was barely utilized, if at all.

2. Growth occurred on media providing the following nitrogen sources when the carbon source was sucrose: sodium nitrate, ammonium tartrate, calcium nitrate, ammonium chloride, ammonium sulfate, asparagine, and glycine.

3. These findings are not in complete agreement with previous reports on nutrition of *Apodachlya brachynema*. Consequently certain interpretations based on physiological characteristics which have been re-

garded as of phylogenetic significance would seem to require reconsideration.

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STUDIES ON TODDY YEAST

MAJEED AHMAD, A. RAZZAQUE CHAUDHURY AND K. U. AHMAD

(WITH 3 FIGURES)

Toddy from the Palmyra palm, *Borassus flabellifer,* is an important beverage in the Indo-Pakistan sub-continent and it was therefore decided to study the yeast responsible for fermenting the juice of this palm.

Studies on the fermentation of palm juices have been carried out by various workers in other countries. Dade,[1] in the Gold Coast, Africa, isolated *Saccharomyces cerevisiae, Candida krusei, Candida mycoderma, Kloeckera apiculata, Schizosaccharomyces pombe* and *Hansenula* sp. from the toddy of the oil palm, *Elaies guineensis.* Guilliermond (**1914**) has mentioned the isolation of *Saccharomyces chevalieri* also from the juice of this palm. Reyne [1] isolated *Saccharomyces chevalieri* from the juice of the Arenga palm, *Arenga saccharifera.* Roelfsen [1] isolated also from the juice of the same palm, *Saccharomyces cerevisiae.* Saito and Otani (**1936**), in Japan, reported from palm juice the isolation of *Endomycopsis monosporous* (now *Endomycopsis fibuliger* var. *monospora*), *Schizosaccharomyces pombe, Saccharomycodes ludwigii, Pichia membranaefaciens, Zygopichia chevalieri* var. *fermentati* (now *Pichia farinosa*). Saito and Otani in **1935** also reported the isolation of *Kloeckera japonica* (now *Kloeckera apiculata*) from palm juice. Guilliermond (**1914**) has mentioned the isolation of *Saccharomyces mangini* (now *Saccharomyces chevalieri*) from Biliwine (wine from the juice of *Osbeckia grandiflora*).

MATERIAL AND METHODS

Thirteen cells were isolated from two samples of toddy from the palmyra palm, *Borassus flabellifer,* with the help of a micro-manipulator, and single-cell cultures were grown in a germination medium after C. Lindegren, Spiegelman and G. Lindegren (**1944**). It has the following composition per liter:

[1] Personal communication from Miss Genderen, Delft.

Autolysed yeast extract powder	2.0 gms
Ammonium sulphate	1.0 gm
Potassium dihydrogen phosphate	1.866 gms
Calcium chloride	0.125 gm
Bacto peptone	3.0 gms
Potassium phosphate	0.133 gm
Magnesium sulphate	0.25 gm
50% sodium lactate	1.0 cc

When the medium was required for use, 4 gms of glucose and 0.75 gm of agar were added to every 100 cc of the medium.

The single cells that gave viable progenies were isolated in a standard medium which has the following composition per liter:

Peptone	3 gms
Asparagine	1 gm
Potassium dihydrogen phosphate	2 gms
Magnesium sulphate	1 gm
Hydrated calcium chloride ($CaCl_2 \cdot 6H_2O$)	0.5 gm
Ammonium sulphate	2.0 gms
Potassium iodide	0.1 gm
Yeast extract	1 gm
Distilled water	1000 cc

Trace elements were added in the following proportion:

Sodium borate	4.3 gms
Manganese chloride	0.1 gm
Zinc sulphate	1.5 gms
Copper sulphate	0.1 gm
Sodium molybdate	0.1 gm
Ferrous sulphate	1.2 gms

Vitamins were added in gammas per liter of medium as follows:

Thiamin	200 gammas
Riboflavin	100 gammas
Pyridoxin	200 gammas
Nicotinic acid	200 gammas
Pantothenic acid	200 gammas
Inositol	1000 gammas
Biotin	2 gammas

When the solution was required for use, 4 gms of glucose was added to every 100 cc.

Tests for sporulation capacity of strains or single spore cultures from them were made on nutrient agar. Whenever it was desired to isolate spores from a strain, it was sub-cultured in a modified Lindgren's presporulation medium for 72 hours and then poured on sterile gypsum blocks kept in sterile water acidified to pH 4. Lindegren's modified presporulation medium had the following composition per 200 cc.

Beet root extract	40 cc
Beet leaf extract	20 cc
Raisin extract	103 cc
Dried yeast	0.5 gm

Single spore cultures were tested for mating reaction in 2% liquid malt. Capacities of yeasts for fermenting various sugars were tested by first subculturing the yeasts for 48 hours. They were next washed in sterile water and inoculated in a test tube containing 15 cc. of standard medium with 2% of the sugar to be tested and an inverted Durham tube. Tests were made in duplicate and readings were taken after 48 hours.

Biochemical deficiencies of yeast were tested in a synthetic medium after Burkholder, McVeigh and Moyer (**1944**). Tests were made in duplicate and readings were taken after 72 hours.

Giant colonies were made on malt-glucose-agar plates and characters of giant colonies were recorded after 3 weeks. The malt-glucose-agar medium had the following composition:

Malt	4 gms
Glucose	2 gms
Agar	5 gms
Distilled water	100 cc

EXPERIMENTAL RESULTS

Of the thirteen isolants, eleven proved to be pure cultures whereas two were found to be mixed. The two mixed types were replated and two pure cultures were obtained from each of them. Thus, in all, fifteen yeast cultures were obtained.

Studies on cell shape, method of vegetative muliplication and capacity to form spores showed that the fifteen isolants from toddy fell into three genera: *Schizosaccharomyces, Saccharomyces,* and *Saccharomycodes.* Their characteristics and taxonomic position is as follows:

GENUS *Schizosaccharomyces:* Eight of the isolants proved to be *Schizosaccharomyces pombe* Lindner. They have cylindrical cells but form no pseudo-mycelium. Vegetative multiplication is by means of transverse fission. They form 2- or 4-spored asci. Asci mostly linear, a few dumbbell-shaped. They fermented sucrose, maltose, α-methyl glucoside, and 1,3-raffinose but failed to ferment galactose, melibiose and lactose. The giant colonies from them were either white, circular, smooth, low-convex, margin crenate (FIG. 1A) or buff, circular, opaque, smooth, raised with concave bevelled edge, radially plicate, margin crenate (FIG. 1B). When tested for their vitamin synthesis capacities

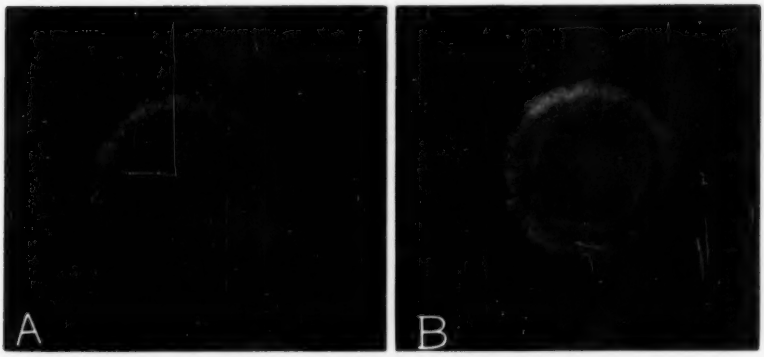

FIG. 1. *Schizosaccharomyces pombe*. Giant colonies on malt-glucose-agar. A. T_1, B. T_2B. ×3.

all eight strains of this yeast proved to be heterotrophic and lacked the capacity to synthesize inositol (TABLE I). Six strains synthesised riboflavin, thiamin, biotin and pyridoxin but failed to synthesise pantothenic acid either completely (T_2, T_{10}, T_{11}, T_{12}, T_{13}) or almost completely (T_1). The remaining two strains of this yeast differed from the other six in being synthesisers of pantothenic acid and complete or almost complete non-synthesisers of biotin. These two strains alsc failed to grow in all vitamin media.

TABLE I

VITAMIN SYNTHESIS CAPACITIES OF THE 15 ISOLANTS FROM TODDY

Species	Strains	Growth in synthetic media								
		Basal solution	Pantothenic-less	Riboflavinless	Thiaminless	Niacinless	Inositolless	Biotinless	Pyridoxinless	All vitamins
Schizosaccharomyces pombe	T_1	−	−(t)	+	+	+(l)	−	+	+	+
	T_2, T_{10}	−	−	+	+	+(l)	−	+	+	+
	T_{11}, T_{12}, T_{13}	−	−	+	+	−(t)	−	+	+	+
	T_3B	−	−	+(l)	+(l)	+(t)	−	−(t)	+(l)	−
	T_9B	−	+	+(l)	+(l)	−	−	−	+	−
Saccharomycodes ludwigii	$T_{15}A$	+	+	+	+	+	+	+	+	+
Saccharomyces chevalieri	T_4, T_5, T_6, T_7, T_8	+	+	+	+	+	+	+	+	+
	T_9A	+(l)	+	+(l)	+	+	+	+	+	+

\+ = Normal growth.
− = No growth.
+(l) = Growth less than normal.
−(t) = Negligible growth.

TABLE II

VITAMIN SYNTHESIS CAPACITIES OF SINGLE-SPORE CULTURES FROM STRAIN T_1 OF SCHIZOSACCHAROMYCES POMBE

Single-spore culture	Growth in synthetic media								
	Basal solution	Panto-thenic-less	Ribofla-vinless	Thiamin-less	Niacin-less	Inositol-less	Biotin-less	Pyri-doxin-less	All vitamins
1a	−	−	+	+	−	−	+	+	+
1b	−	−	+	+	−	−	+	+	+
1c	−	−	+	+	−	−	+	+	+
1d	−	−	+	+	−	−	+	+	+

In order to see whether these yeasts were homothallic or heterothallic and also whether they were heterozygous or homozygous for the genes controlling the synthesis of various members of the vitamin B complex, ten asci were dissected from one of the strains, T_1. Four spores from an ascus germinated only in the case of three asci and when these twelve single-spore cultures were tested for mating reaction, they all showed the capacity to self-diploidise and mate in all combinations. This strain of *Schizosaccaromyces pombe* is thus homothallic.

Four single ascospore cultures from ascus 1 of T_1 were tested for their vitamin synthesis capacities. These tests showed homozygosity of genes for synthesis of riboflavin, thiamin, biotin and pyridoxin and also the homozygosity of genes for the non-synthesis of pantothenic acid, nicotinic acid, and inositol (TABLE II).

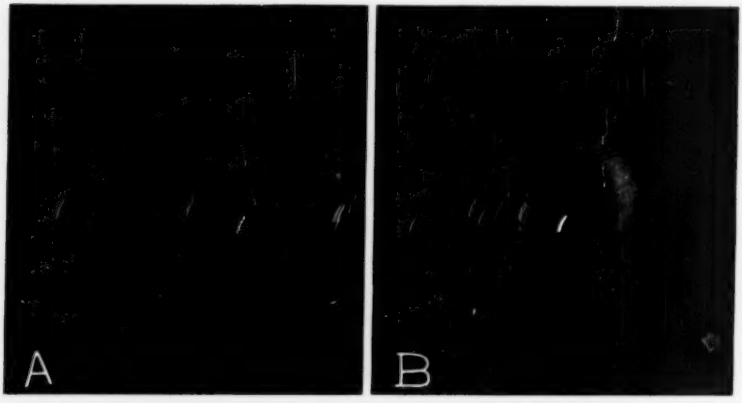

FIG. 2. *Saccharomyces chevalieri*. Giant colonies on malt-glucose-agar. A. T_1, B. T_7. × 3.

GENUS *Saccharomyces:* Six of the isolants from toddy proved to be *Saccharomyces chevalieri* Guilliermond. They have round or oval cells and multiply by budding. They usually form 2- and 4-spored asci more or less spherical or rhomboidal in shape. They ferment sucrose, galactose and raffinose but fail to ferment maltose, lactose, α-methyl glucoside, and melibiose. The giant colonies from them are either white,

TABLE III

SPORULATION CAPACITY AND MATING REACTION OF 16 SINGLE-SPORE CULTURES FROM 4 ASCI OF STRAIN T_7 OF SACCHAROMYCES CHEVALIERI

Ascus No.	Single-spore culture	Sporulation capacity	Mating behavior with the tester strains from yeast foam		Mating type of the culture
			With (+)	With (−)	
2	2a	×	No reaction	Gametes and zygotes common	(+)
	2b	Present	Gametes rare	Gametes rare	?
	2c	×	No reaction	Gametes very common	(+)
	2d	Present	Gametes and zygotes common	No reaction	(−)
3	3a	×	No reaction	No reaction	?
	3b	Present	Rare stimulated cells	No reaction	(−)
	3c	×	No reaction	Rare stimulated cells	(+)
	3d	×	No reaction	Gametes and zygotes seen	(+)
4	4a	×	No reaction	Few stimulated cells	(+)
	4b	×	No reaction	Gametes found	(+)
	4c	Present	Rare stimulated cells	No reaction	(−)
	4d	Present	No reaction	No reaction	?
5	5a	Present	Gametes seen	No reaction	(−)
	5b	×	No reaction	Gametes and zygotes common	(+)
	5c	Present	Gametes rare	No reaction	(−)
	5d	×	No reaction	Gametes common	(+)

circular, opaque, raised, radially plicate, margin lobate (FIG. 2A) or white, circular, opaque, surface slightly rough, raised with concave bevelled edge, radially plicate, margin lobate (FIG. 2B). When tested for their vitamin synthesis capacities they proved to be autotrophic. One of the strains T_3A, showed, however, a subnormal synthesis in the basal and riboflavinless medium (TABLE I).

In order to see whether these yeasts were homothallic or heterothallic and also whether they were heterozygous or homozygous for the genes

controlling fermentation of sugars and synthesis of the various members of vitamin B complex, eighteen four-spored asci from strain T_5, ten asci from strain T_6 and six asci from strain T_7 of this yeast were dissected. All four spores germinated to give viable colonies from four asci from each strain. When the sixteen single-spore cultures from each of the three strains were tested for mating reaction and sporulation capacity, strains T_5 and T_6 proved to be homothallic as single spore cultures from them mated in all combinations, self diploidised and usually formed spores. The situation in T_7 proved to be complicated. It was neither clear cut homothallic nor heterothallic.

The data incorporating studies on sporulation capacity and mating reaction with the (+) and (−) tester strains from Yeast Foam (TABLE III) suggest that this yeast is probably basically heterothallic as two single ascospore cultures from each ascus proved to be (+). The two

TABLE IV

SEGREGATION RATIOS AMONGST THE 4 SPORES FROM THE SAME ASCUS FOR FERMENTATION OF MALTOSE, GALACTOSE, AND RAFFINOSE IN STRAINS T_5 AND T_7 OF SACCHAROMYCES CHEVALIERI

Strain	Number of asci	Ratio of fermenters to non-fermenters		
		Maltose	Galactose	Raffinose
T_5	2	0:4	2:2	4:0
T_7	3	0:4	2:2	4:0
	1	1:3	2:2	4:0

single ascospore cultures from each ascus which were expected to be (−) on the basis of a heterothallic mating system, were in most cases interfered with. Thus 3b, 4c and 5c gave a weak (−) reaction while 3a and 4d gave no mating reaction. Further, of the eight expected (−) single ascospore cultures from these four asci, seven formed ascospores.

Eight single ascospore cultures from T_5 and sixteen single ascospore cultures from T_7 were tested for their fermentation capacities of maltose, galactose and raffinose. As shown in TABLE IV, like the parent, all the eight single ascospore cultures from T_5 lacked the capacity to ferment maltose but fermented raffinose. With regard to the fermentation of galactose, it was found that two single ascospore cultures from each ascus fermented it, while two from each ascus failed to do so. This strain of *Saccharomyces chevalieri* thus proved to be homozygous for the non-fermenting maltose genes and the genes fermenting raffinose. It, however, proved to be heterozygous for galactose fermentation genes.

In the case of T_7, fifteen single ascospore cultures failed to ferment maltose but one fermented it (TABLE IV). This single spore culture apparently shows a mutation of the non-fermenter to the fermenter allele of maltose. With regard to the fermentation of galactose, two single ascospore cultures from each ascus proved to be fermenters and two non-fermenters. Thus like T_5, this strain is homozygous for maltose non-fermenting genes and raffinose-fermenting genes but heterozygous for the galatose fermenting genes.

Sixteen single ascospore cultures from each of the strains T_5 and T_7 were tested for their capacities to synthesize various members of the vitamin B complex (TABLE V). In T_5, loci for the synthesis of panto-

TABLE V

SEGREGATION RATIOS AMONGST THE 4 SPORES FROM THE SAME ASCUS FOR SYNTHESIS OF VARIOUS MEMBERS OF VITAMIN B COMPLEX IN STRAINS T_5 AND T_7 OF SACCHAROMYCES CHEVALIERI

Strains	Number of asci	Ratio of synthesisers to non-synthesisers						
		Panto-thenic-less	Ribofla-vinless	Thiamin-less	Niacin-less	Inositol-less	Biotin-less	Pyri-doxin-less
T_5	2	4:0	4:0	4:0	4:0	3:1	4:0	4:0
	1	4:0	4:0	4:0	4:0	4:0	3:1	4:0
	1	4:0	4:0	4:0	4:0	4:0	4:0	4:0
T_7	2	3:1	4:0	4:0	4:0	4:0	2:2	4:0
	1	4:0	4:0	4:0	4:0	4:0	2:2	4:0
	1	3:1	4:0	4:0	4:0	1:3	2:2	4:0

thenic acid, riboflavin, thiamin, niacin, and pyridoxin were found to be homozygous for the synthesiser genes, as a 4:0 segregation was shown by all the four asci for them. In the case of inositol synthesis, two asci showed a 3:1 segregation while the other two a 4:0 segregation. In the case of biotin synthesis one ascus showed a 3:1 segregation and three asci a 4:0 segregation.

Vitamin synthesis tests on sixteen single-ascospore cultures from four asci belonging to T_7 showed the homozygosity of this yeast for the genes concerned in the synthesis of riboflavin, thiamin, niacin, and pyridoxin (TABLE V). Three asci showed a 3:1 and one a 4:0 segregation of synthesisers of pantothenic acid to non-synthesisers. With respect to inositol synthesis, three asci showed a 4:0 segregation and one a 1:3 segregation of synthesisers to non-synthesisers. Segregation for the biotin locus was found to be 2:2 in all the four asci.

GENUS *Saccharomycodes:* Of the fifteen isolants, only one proved to be *Saccharomycodes ludwigii* Hansen. It has lemon-shaped cells with flat poles and multiplies vegetatively by terminal budding followed by septation. It does not form any spores. It ferments sucrose. It does not ferment maltose, galactose, raffinose, lactose, and α-methyl glucoside. Its giant colony was brownish, circular, opaque, low-convex, with a crater in the middle, radially plicate, ends of the folds translucent towards the periphery, margin lobate (FIG. 3). It is autotrophic with regard to the synthesis of various members of vitamin B complex (TABLE I).

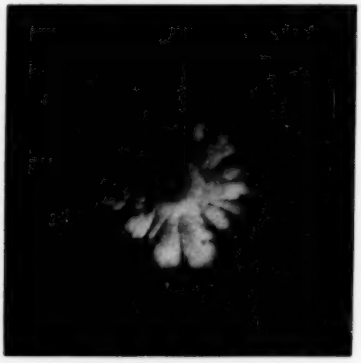

FIG. 3. *Saccharomycodes ludwigii.* Giant colony on malt-glucose-agar. × 3.

DISCUSSION

Fifteen yeasts isolated from toddy obtained from Palmyra palm, *Borassus flabellifer,* fell into three genera and three species. Eight belonged to *Schizosaccharomyces pombe* Lindner, six to *Saccharomyces chevalieri* Guilliermond, and one to *Saccharomycodes ludwigii.* The majority of the yeasts fermenting the juice of this palm thus belong to *Schizosaccharomyces pombe* and *Saccharomyces chevalieri. Saccharomycodes ludwigii* seems to be much rarer. Of the above three yeasts, Dade isolated only *Schizosaccharomyces pombe* from the toddy of the oil palm, *Elaies guineensis.* Saito and Otani (**1936**) recovered not only *Schizosaccharomyces pombe* but also *Saccharomycodes ludwigii* from palm juice. *Saccharomyces chevalieri* has been found to be more common in the palm juices. Thus Reyne isolated it from the juice of Arenga palm, *Arenga saccharifera,* while Guilliermond (**1914**) has reported its isolation from the toddy of the oil palm, *Elaies guineensis,* and from the wine of the juice of *Osbeckia grandiflora.* All three yeasts

isolated by the authors from the toddy thus seem to be world-wide in their association with the natural fermentation of the palm juices.

Strains of *Schizosaccharomyces pombe* isolated by us formed both linear and dumbbell-shaped asci. Their vegetative phase thus consisted of both diploid and haploid cells. They conformed to the standard strains of this yeast in their fermentation capacities, fermenting sucrose, maltose and raffinose (⅓) but failing to ferment galactose, melibiose and lactose, as has been mentioned by Lindegren (**1949**) and Lodder and Kreger-Van Rij (**1952**). Vitamin synthesis studies on the eight isolants of this yeast showed that they were all heterotrophic and deficient in the synthesis of inositol (TABLE I). Synthesis of nicotinic acid was found to be poor or subnormal in all of them. Six strains were found to be completely or almost completely deficient in the synthesis of pantothenic acid. The remaining two strains synthesised pantothenic acid but were deficient in the synthesis of biotin.

Burkholder and Moyer (**1943**) found one strain of *Schizosaccharomyces pombe* to be deficient in biotin, pantothenic acid, nicotinic acid and inositol while another strain of this yeast was reported to be deficient in pyridoxin and thiamin by Burkholder, McVeigh and Moyer (**1944**). It is thus seen that strains of this yeast can be deficient for any member of the vitamin B complex except riboflavin. Deficiences for inositol, pantothenic acid, nicotinic acid and biotin seem to be more common than those for thiamin and pyridoxin. It is noteworthy that two strains of this yeast (T_3B and T_9B, TABLE I) failed to grow in medium containing all seven members of the vitamin B complex but grew normally in some media where only six members of this group of vitamins were present.

When one of the strains of *Schizosaccharomyes pombe*, T_1, was tested for its mating system, it was found to be homothallic. Leupold (**1950**) investigated a culture of *Schizosaccharomyces pombe*, str. *liquefaciens*, from Delft (Holland) and found it to be a mixture of two homothallic strains of different fertilities, two mating types of a heterothallic strain and a sterile strain. No evidence for heterothallism or a mutation to sterility was found by the authors of strain T_1 of this yeast.

No report has been found by the authors on homozygosity or heterozygosity of *Schizosaccharomyces pombe* for the vitamin synthesis genes. As shown in TABLE II, the strain T_1 of this yeast was found to be homozygous for the genes synthesising riboflavin, thiamin, biotin and pyridoxin.

All the six strains of *Saccharomyces chevalieri* Guilliermond examined by the authors proved to be non-fermenters of maltose but fermenters

of galactose. Their lack of fermentation of maltose is in accordance with the finding of previous workers (Stelling-Dekker **1931**, Winge and Roberts **1948**). Fermentation of galactose by them within 48 hours was unexpected, for according to Stelling-Dekker (**1931**) this yeast is a non-fermenter of galactose whereas Winge and Roberts (**1948**) found that it fermented this sugar only in 8–10 days. Lodder and Kreger-Van Rij, however, state that it normally ferments galactose and it is only occasionally that it fails to ferment this sugar.

Tests on single-spore cultures from T_5 and T_7 proved that these strains were homozygous for maltose non-fermenters and raffinose fermenters (TABLE IV) but heterozygous for fermentation of galactose. One ascospore from T_7 proved to be a fermenter of maltose and shows apparently a case of mutation of maltose non-fermenter gene to the fermenter allele.

Vitamin synthesis tests revealed that the six strains of *S. chevalieri* are autotrophic. Tests on single ascospore cultures showed that strains T_5 and T_7 were homozygous for the synthesis of riboflavin, thiamin, niacin and pyridoxin (TABLE V). Strain T_5 was found to be homozygous, while strain T_7 was heterozygous for the synthesis of patothenic acid. As in T_7, three asci showed a 3:1 and one a 4:0 segregation of synthesisers to non-synthesisers; more than one locus seems to be concerned in the synthesis of pantothenic acid in this strain. Strain T_5 showed 3:1 and 4:0 segregations for the synthesis of inositol and biotin. These 3:1 and 4:0 segregations for inositol and biotin seem to be due to more than one locus being concerned in the synthesis of inositol and biotin and the heterozygosity of these loci, or due to the mutation of synthesiser alleles to non-synthesiser. Segregation for inositol synthesis in the strain T_7 was found to be anomalous as three asci gave a 4:0 segregation whereas one ascus a 1:3 segregation. Synthesis of biotin in this strain was found to be controlled by a single locus, which was heterozygous, as all the four asci gave a 2:2 segregation of biotin synthesisers to non-synthesisers.

Investigations on the mating system of three strains of *Saccharomyces chevalieri* showed that two of them (T_5 and T_6) were homothallic. Analysis of sixteen single ascospore cultures from four asci belonging to the third strain, T_7, showed that two spores from each ascus were (+). Of the remaining eight cultures which were expected to be (−) on a heterothallic scheme, five showed a strong or weak (−) mating reaction but in three cases (2b, 3a and 4d) no decision could be made. When all the sixteen single ascospore cultures were tested for sporulation, none of the (+) cultures were found sporing, but of the expected eight (−)

cultures, seven formed ascospores. This showed that these seven cultures had self-diploidised. This may be due to a high mutation rate of the (−) allele to (+) or due to the close linkage of a self diploidising gene to the (−) allele.

Winge and Roberts (**1949**) reported that *Saccharomyces chevalieri* is homothallic and homozygous for a gene controlling self-diploidisation. The investigations of the authors showed that while the majority of strains of this yeast are homothallic, a few may be basically heterothallic with selfing superimposed either through the mutation of the mating type alleles or the presence of a self-diploidising gene.

According to previous reports (Lindegren, **1949**, and Lodder and Kreger-Van Fij, **1952**) *Saccharomycodes ludwigii* ferments ⅓ raffinose. The only peculiarity shown by the strain of this yeast isolated by us is that it failed to ferment raffinose.

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The authors are much indebted to Miss W. Slooff and Miss A. V. D. Hoven van Genderen of the Centraalbureau Voor Schimmelcultures, Delft (Holland) for the identification of the strains.

SUMMARY

1. Fifteen cultures of yeast were isolated from the toddy obtained from palmyra palm, *Borassus flabellifer*.

2. Eight of the cultures belonged to *Schizosaccharomyces pombe* Lindner, six to *Saccharomyces chevalieri Guilliermond* and one to *Saccharomycodes ludwigii* Hansen.

3. *Schizosaccharomyces pombe* strains were deficient in the synthesis of inositol and poor or subnormal in the synthesis of nicotinic acid. Six of them were completely or almost completely deficient in the synthesis of pantothenic acid. The remaining two strains synthesised pantothenic acid but were deficient in biotin.

4. One of the strains of *Schizosaccharomyces pombe* analysed for its mating system proved to be homothallic. It also proved to be homozygous for the genes concerned in the synthesis of riboflavin, thiamin, biotin, and pyridoxin.

5. All the six strains of *Saccharomyces chevalieri* fermented galactose in 48 hours. Analysis of single ascospore cultures from two of them showed that they were heterozygous for the fermentation of this sugar.

6. A case of mutation from maltose non-fermenter allele to the fermenter allele was recorded in one of the strains of *Saccharomyces chevalieri*.

7. All the six strains of *Saccharomyces chevalieri* proved to be autotrophic with respect to the synthesis of various members of vitamin B complex. Two of the strains were homozygous for the synthesis of riboflavin, thiamin, nicotinic acid and pyridoxin. Synthesis of biotin in one of the strains was found to be controlled by a single locus for which it was heterozygous.

Two strains of *S. chevalieri* proved to be homothallic and one seemed to be basically heterothallic.

8. The strain of *Saccharomycodes ludwigii* isolated, failed to ferment raffinose.

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DIFFERENTIATION OF SPECIES OF TAPHRINA IN CULTURE. UTILIZATION OF CARBON COMPOUNDS

A. J. Mix

In a recent paper (**1**) an account was given of the utilization of various nitrogen compounds by certain isolates of *Taphrina*. The behavior of most of these isolates with regard to utilization of carbon compounds will be reported in the present paper.

The list of isolates and their sources as given earlier (l.c.) will not be repeated. The following cultures were not used: Nos. *65, 66, 68* (*Taphrina robinsoniana*); Nos. *72, 73* (*T. sadebeckii*). The non-sporulating yeasts (certain species of *Candida, Cryptococcus,* and *Torulopsis*) included in the earlier (**1**) experimentation were also omitted.

All cultures were made in duplicate in 15 × 125 mm tubes, each tube containing 4.5 ml of liquid medium. For most of the cultures this medium was: NH_4Cl 1.1 gm, KH_2PO_4 2.75 gm, $MgSO_4 \cdot 7H_2O$ 1.25 gm, carbon compound 1 gm, distilled water 1000 ml. The medium used for *Taphrina caerulescens* from *Quercus macrocarpa* (culture No. *11*) and from *Q. prinoides* (culture No. *17*) contained NH_4NO_3, 0.8 gm per liter in the place of NH_4Cl. Each complete medium was used with and without the addition of thiamine hydrochloride, one part per million.

The initial hydrogen-ion concentration of the medium was usually near pH 4.5 though it ranged between pH 4.2 and pH 5.2.

Cultures were kept at room temperature (usually 20°–24° C) and observations were made at the end of 21 days. Growth was estimated by visual observation of the degree of turbidity of the medium, three categories being recognized: (a) no growth, (b) slight but definite growth, (c) moderate to good growth.

On the first trial all of the cultures used dextrose, sucrose, maltose, melezitose, trehalose, dextrin, inulin, and pectin, and all of them refused lactose, rhamnose, inositol, and i-erythritol. These experiments were not repeated. With the other carbon compounds tested each case of refusal of a particular compound by a particular culture was made the subject of a second trial. A number of repetitions were also made with KNO_3, 2 gm per liter as the source of nitrogen.

An account follows of the utilization by individual cultures of the

carbon compounds tested (other than those listed above). Slight utilization is indicated by means of a parenthesis enclosing the compound in question.

Taphrina americana from *Betula fontinalis*, culture No. 1, used: galactose, levulose, mannose, raffinose, l-arabinose, xylose, (adonitol), (dulcitol), mannitol, d-sorbitol, citric acid, succinic acid.

T. betulina from *B. intermedia*, culture No. 2, used: levulose, melibiose, l-arabinose, xylose, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. betulina from *B. intermedia* (formerly *T. lapponica*), culture No. 3, used: galactose, levulose, mannose, (cellobiose), melibiose, l-arabinose, xylose, mannitol, d-sorbitol, glycerol, (soluble starch), citric acid, succinic acid.

T. betulina from *B. pendula* (formerly *T. turgida*), culture No. 4, used: (galactose), levulose, mannose, (raffinose), xylose, mannitol, (d-sorbitol), (soluble starch), citric acid, succinic acid.

T. caerulescens from *Quercus alba*, culture No. 5, used: levulose, mannose, cellobiose, (d-ribose), xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, succinic acid.

T. caerulescens from *Q. bushii*, culture No. 6, used: (galactose), levulose, mannose, cellobiose, (melibiose), l-arabinose, d-ribose, (adonitol), dulcitol, mannitol, d-sorbitol, xylose, glycerol, salicin, succinic acid.

T. caerulescens from *Q. coccinea*, culture No. 7, used: levulose, mannose, (raffinose), cellobiose, l-arabinose, d-ribose, (l-sorbose), xylose, adonitol, dulcitol, mannitol, d-sorbitol, glycerol, ethanol, salicin, citric acid, succinic acid.

T. caerulescens from *Q. ilicifolia*, culture No. 8, used: levulose, mannose, cellobiose, melibiose, xylose, adonitol, dulcitol, mannitol, d-sorbitol, glycerol, ethanol, salicin, citric acid, succinic acid.

T. caerulescens from *Q. kelloggii*, culture No. 9, used: levulose, mannose, cellobiose, d-ribose, (l-sorbose), xylose, adonitol, dulcitol, mannitol, d-sorbitol, glycerol, ethanol, salicin, succinic acid.

T. caerulescens from *Q. laurifolia*, culture No. 10, used: levulose, mannose, (raffinose), cellobiose, melibiose, d-ribose, l-sorbose, xylose, (adonitol), mannitol, d-sorbitol, glycerol, salicin, succinic acid.

T. caerulescens from *Q. macrocarpa*, culture No. 11, used: levulose, mannose, l-arabinose, xylose, (adonitol), dulcitol, (mannitol), (d-sorbitol), glycerol, (ethanol), soluble starch, citric acid, succinic acid.

T. caerulescens from *Q. marilandica*, culture No. 12, used: galactose, levulose, mannose, l-arabinose, cellobiose, xylose, (adonitol), mannitol, d-sorbitol, (ethanol), salicin, citric acid, succinic acid.

T. caerulescens from *Q. maxima*, cultures No. 13 and No. 14, used: galactose, levulose, mannose, raffinose, l-arabinose, melibiose, d-ribose, l-sorbose, xylose, adonitol, dulcitol, mannitol, d-sorbitol, glycerol, ethanol, citric acid, succinic acid.

T. caerulescens from *Q. nigra*, culture No. 15, used: (galactose), levulose, (raffinose), cellobiose, (l-sorbose), xylose, (adonitol), mannitol, d-sorbitol, glycerol, salicin, succinic acid.

T. caerulescens from *Q. palustris*, culture No. 16, used: levulose, galactose, mannose, (raffinose), l-arabinose, cellobiose, d-ribose, xylose, adonitol, dulcitol, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. caerulescens from *Q. prinoides*, culture No. 17, used: levulose, galactose, mannose, l-arabinose, (melibiose), l-sorbose, xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. caerulescens from *Q. velutina*, cultures No. 18 and No. 19, used: levulose, galactose, mannose, (raffinose), l-arabinose, cellobiose, d-ribose, xylose, adonitol, dulcitol, mannitol, d-sorbitol, glycerol, ethanol, salicin, soluble starch, citric acid, succinic acid.

T. caerulescens from *Q. virginiana*, culture No. 20, used: mannose, cellobiose, (d-ribose), xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. carnea from *Betula intermedia*, culture No. 21, used: levulose, (galactose), mannose, raffinose, cellobiose, l-arabinose, xylose, adonitol, (mannitol), d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. cerasi from *Prunus avium*, cultures No. 22, No. 23, and No. 24, used: levulose, mannose, raffinose, cellobiose, l-sorbose, xylose, adonitol, mannitol, d-sorbitol, salicin, citric acid, succinic acid.

T. cerasi from *P. pennsylvanica*, cultures No. 25 and No. 26, used: levulose, galactose, mannose, l-arabinose, cellobiose, d-ribose, l-sorbose, xylose, mannitol, d-sorbitol, glycerol, citric acid, succinic acid.

T. communis from *P. americana*, cultures No. 27, No. 28, and No. 29, used: levulose, galactose, mannose, raffinose, l-arabinose, cellobiose, d-ribose, l-sorbose, xylose, adonitol, mannitol, d-sorbitol, glycerol, citric acid, succinic acid.

T. communis from *P. angustifolia*, cultures No. 30, No. 31, and No. 32, used: levulose, galactose, mannose, raffinose, cellobiose, melibiose, l-sorbose, xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, soluble starch, citric acid, succinic acid.

T. communis from *P. lanata*, culture No. 33, used: levulose, mannose, raffinose, cellobiose, (melibiose), (d-ribose), l-sorbose, xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. communis from *P. maritima*, culture No. 34, is used: levulose, mannose, raffinose, (l-arabinose), cellobiose, melibiose, d-ribose, l-sorbose, xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. communis from *P. nigra*, culture No. 35, used: galactose (with KNO_3), levulose, mannose (with KNO_3), raffinose, l-arabinose (with KNO_3), cellobiose, d-ribose, l-sorbose, xylose, adonitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. communis from *Prunus* sp., cultures No. 36 and No. 37, used: galactose (with KNO_3), levulose, mannose, raffinose, (l-arabinose), cellobiose, melibiose, d-ribose, l-sorbose, xylose, adonitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. confusa from *P. virginiana*, culture No. 38, used: (galactose), levulose, mannose, raffinose, cellobiose, xylose, adonitol, (dulcitol), d-sorbitol, glycerol, salicin, soluble starch, citric acid, succinic acid.

T. confusa from *P. virginiana* var. *demissa*, culture No. 39, used: galactose, levulose, mannose, raffinose, l-arabinose, cellobiose, melibiose, d-ribose, xylose, adonitol, dulcitol, d-sorbitol, glycerol, salicin, soluble starch, citric acid, succinic acid.

T. dearnessii from *Acer rubrum*, culture No. 40, used: galactose, mannose, raffinose, l-arabinose, cellobiose (with KNO_3), melibiose, xylose, adonitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. deformans from *P. persica*, culture No. 41, and from *P. communis*, culture No. 42, used: l-sorbose, xylose, adonitol, d-sorbitol, glycerol, (salicin), citric acid, succinic acid.

T. epiphylla from *Alnus incana*, cultures No. 43 and No. 44, used: galactose, levulose, mannose, raffinose, cellobiose, xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. farlowii from *P. serotina*, cultures No. 45 and No. 46, used: mannose, raffinose, cellobiose, (melibiose), xylose, adonitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. flavorubra from *P. pumila* var. *susquehanae*, culture No. 47, used: levulose, (mannose), raffinose, cellobiose, (d-ribose), xylose, (adonitol), d-sorbitol, glycerol, (salicin), citric acid, succinic acid.

T. flavorubra from *P. besseyi*, culture No. 48, used: levulose, mannose, raffinose, cellobiose, (d-ribose), xylose, adonitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. johansonii from *Populus tremuloides*, cultures No. 49 and No. 50, used: levulose, mannose, cellobiose, xylose, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. letifera from *Acer spicatum*, culture No. 51, used: levulose, raffinose, cellobiose, xylose, adonitol, mannitol, (d-sorbitol), glycerol, (salicin), citric acid, succinic acid.

T. nana from *Betula nana*, culture No. 52, used: galactose, levulose, mannose, raffinose (with KNO_3), l-arabinose, (d-ribose), xylose, (adonitol), (d-sorbitol), soluble starch (with KNO_3), citric acid.

T. polystichi from *Polystichum acrostichoides*, cultures No. 53 and No. 54, used mannitol.

T. populina from *Populus nigra*, cultures No. 55 and No. 56, used: levulose, mannose, cellobiose, xylose, mannitol, d-sorbitol, (glycerol), salicin, succinic acid.

T. populi-salicis from *P. fremontii*, culture No. 57, used: levulose, mannose, cellobiose, xylose, mannitol, d-sorbitol, salicin, succinic acid.

T. populi-salicis from *P. trichocarpa*, culture No. 58, used: levulose, mannose (with KNO_3), cellobiose (with KNO_3), xylose, mannitol, d-sorbitol, succinic acid.

T. potentillae from *Potentilla arguta*, culture No. 59, used: galactose (with KNO_3), levulose, mannose (with KNO_3), raffinose, (cellobiose), xylose, mannitol, d-sorbitol, (salicin), soluble starch, succinic acid.

T. potentillae from *P. canadensis*, cultures No. 60 and No. 61, used: levulose, mannose, raffinose, l-arabinose, cellobiose, d-ribose, xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. pruni-subcordatae from *Prunus subcordata*, culture No. 62, used: galactose, levulose, mannose, raffinose, l-arabinose, cellobiose, d-ribose, xylose, (adonitol), mannitol, (d-sorbitol), glycerol, ethanol, salicin, succinic acid.

T. purpurascens from *Rhus copallina*, culture No. 63, used: levulose, mannose, raffinose, melibiose, l-sorbose, xylose, mannitol, d-sorbitol, glycerol, salicin, citric acid, succinic acid.

T. robinsoniana from *Alnus rugosa*, culture No. 64, and from *A. serrulata*, cultures No. 67 and No. 69, used: levulose, raffinose, l-arabinose, cellobiose, melibiose, d-ribose, xylose, mannitol, d-sorbitol, glycerol, salicin, soluble starch, citric acid, succinic acid.

T. sacchari from *Acer saccharum*, cultures No. 70 and No. 71, used: galactose, levulose, mannose, raffinose, l-arabinose, cellobiose, melibiose, l-sorbose, xylose, adonitol, mannitol, d-sorbitol, glycerol, ethanol, salicin, soluble starch, citric acid, succinic acid.

T. tosquinetii from *Alnus glutinosa*, cultures No. 74 and No. 75, used: levulose, l-arabinose, xylose, mannitol, d-sorbitol, salicin, succinic acid.

T. ulmi from *Ulmus alata*, culture No. 76, used: galactose, levulose, mannose, l-arabinose, cellobiose, xylose, (adonitol), (dulcitol), manni-

tol, d-sorbitol, glycerol, ethanol, (salicin), soluble starch, citric acid, succinic acid.

T. ulmi from *U. rubra*, cultures No. 77 and No. 78, used: galactose, levulose, mannose, l-arabinose, cellobiose, (l-sorbose), xylose, adonitol, (dulcitol), mannitol, (d-sorbitol), glycerol, ethanol, salicin, soluble starch, citric acid, succinic acid.

T. virginica from *Ostrya virginiana*, culture No. 79, used: galactose, levulose, mannose, l-arabinose, cellobiose, d-ribose, l-sorbose, xylose, adonitol, mannitol, d-sorbitol, glycerol, salicin, soluble starch, succinic acid.

It appears from these data that utilization of carbon compounds may serve as a basis for characterization of the fungi studied. Each species exhibits an individual pattern of carbon utilization. In this respect host-forms within *Taphrina caerulescens* behave like separate species.

Taphrina deformans from *Prunus persica* and from *P. communis* utilize the same carbon compounds; a like agreement exists between *T. flavorubra* from *Prunis pumila* var. *susquehanae* and from *P. besseyi*, between *T. robinsoniana* from *Alnus rugosa* and from *A. serrulata* and between *T. ulmi* from *Ulmus alata* and from *U. rubra*.

Some host forms within species differ only slightly as to carbon compounds utilized: *T. communis* from *Prunus americana, P. angustifolia, P. lanata, P. maritima, P. nigra; T. populi-salicis* from *Populus fremontii* and from *P. trichocarpa*.

Some host forms show greater differences, often as great as those between individual species: the forms of *T. betulina* formerly distinguished as *T. betulina, T. lapponica*, and *T. turgida; T. cerasi* from *Prunus avium* and from *P. pennsylvanica; T. confusa* from *Prunus virginiana* and from *P. virginiana* var. *demissa; T. potentillae* from *Potentilla arguta* and from *P. canadensis*.

An approach to agreement as to carbon compounds utilized exists between the fungi from *Populus: T. johansonii* from carpels of *P. tremuloides, T. populina* from leaves of *P. nigra*, and *T. populi-salicis* from leaves of *P. fremontii* and *P. trichocarpa*.

In these experiments the addition of thiamine hydrochloride to the culture solution induced increased growth of most of the fungi but in no case was its presence necessary to support growth.

SUMMARY

Seventy-four isolates, representing twenty-six species and fifty-five host-forms of *Taphrina*, were grown in mineral solution with thirty-two different carbon compounds. All of these fungi utilized dextrose, su-

crose, maltose, melezitose, trehalose, dextrin, inulin, and pectin. All but one (*T. polystichi*) used xylose and all but two (*T. polystichi* and *T. nana*) used succinic acid. None of them used lactose, rhamnose, inositol, nor i-erythritol. With regard to eighteen compounds: galactose, levulose, mannose, raffinose, cellobiose, l-arabinose, melibiose, l-sorbose, d-ribose, adonitol, dulcitol, mannitol, d-sorbitol, ethanol, glycerol, salicin, soluble starch, and citric acid, each species exhibited an individual pattern of carbon utilization.

Different host-forms of *Taphrina deformans, T. flavorubra, T. robinsoniana,* and *T. ulmi* agreed as to carbon compounds utilized. Other host-forms differed from each other in this respect, sometimes as much as did individual species. There was an approach to agreement between different host-forms of *T. communis* and also between the fungi from *Populus, T. johansonii, T. populina,* and *T. populi-salicis.*

None of these fungi required the addition of thiamine chloride to the medium.

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THE RELATIONSHIP BETWEEN ACTINOMYCES BOVIS AND LACTOBACILLUS BIFIDUS

H. A. Frank and C. E. Skinner

(with 10 figures)

More than once in the history of microbiology, organisms which had been previously widely separated in classification schemes have been found to be very closely related. The present study shows the close relationship between *Actinomyces bovis* Harz and *Lactobacillus bifidus* (Tissier) Holland. Bacteriologists usually consider them as belonging to different orders of the Class Schizomycetes, and there are those, including probably most mycologists, who would classify the former as a true fungus and the latter as a true bacterium.

Actinomyces bovis is a strict anaerobe in the experience of most persons who have actually worked with the species. In any case, it grows with great difficulty, if it grows at all, on surface cultures exposed to the air. It has a tendency to form a primitive branched mycelium which fragments readily into rod shaped bodies which are considered to be arthrospores, and these continue to reproduce by transverse fission. The mycelium is not easily demonstrated for these reasons. If *Actinomyces bovis* is grown in a liquid medium and carefully removed for examination or if it is grown in slide culture it may show the mycelial growth and branching, but at best the mycelium is only rudimentary. It takes bacterial stains and is gram-positive. It is difficult to cultivate and has a tendency to die out in culture. It is found associated with the animal body, either in human or bovine cases of actinomycosis, or in the mouth, teeth, or tonsils of patients or apparently healthy human beings or cattle. Many medical mycologists would separate the organisms isolated from human beings from those isolated from bovine animals, calling the former *Actinomyces israeli*. Since we have made no comparative studies we are here accepting the usual present concept of only one species, although the paper of Erikson (1) and particularly that of Thompson (11) are rather convincing to the contrary. The question of one or two species of the pathogenic anaerobic actinomycetes is further complicated by the fact that they frequently dissociate, so we

may have R or S forms. It is possible that *Actinomyces israeli* will be found to be the S form of *Actinomyces bovis*.

Lactobacillus bifidus is described as a gram-positive rod with a tendency to bifurcation or even to filamentous growth with a slight but definite branching. It is strictly anaerobic and gram-positive. It is difficult to keep in culture and grows best at 37°. It is found in the feces of breast-fed infants (FIG. 10).

From the above, it is evident that there are no characters that would exclude either of these organisms from the other. Puntoni (**7**, **8**) in 1936 and 1937 maintained that the two were identical. The data were few, the serological experiments which confirmed the identity were inconclusive and the conclusions not entirely convincing, but nevertheless the similarity, at least, of the two species was clearly indicated. This rather startling conclusion has been largely ignored. One of the writers (**10**)made a passing reference to it and Slack and associates (**9**) made some preliminary comparative immunological studies in which they found cross agglutinations, but only at very low titers. Negroni and Fischer (**4**) made some careful morphological studies of several strains of *L. bifidus*, and compared them with *A. bovis*. They decided that the two species were distinct but that they belonged to the same genus, *Cohnistreptothrix* Pinoy, a generic name which is, at present, usually considered to be a later synonym of *Actinomyces* Harz.

These following combinations show that some taxonomists have considered *L. bifidus* to be an actinomycete: *Nocardia bifida* Vuillemin 1931 (**12**), *Actinomyces bifidus* Nanizzi in Pollacci (**3**), and *Cohnistreptothrix bifida* Negroni & Fischer 1944 (**4**). Each of these put *A. bovis* in the same genus. Prévot in 1938 (**6**) placed *Bifidobacterium bifidum* (Tissier) Orla-Jensen in the family Actinomycetaceae, but in a different genus from *A. bovis*.

The present study was undertaken to clarify the supposed relationship of *A. bovis* and *L. bifidus*. Although the authors are primarily bacteriologists, nevertheless they believe that morphological characters are, have been, and should be of first importance in separating genera of microorganisms. They had expected to include studies on physiology and serology but the work had to be terminated before much beyond morphological studies was accomplished.

In addition to the two organisms mentioned, there were included cultures of certain "anaerobic diphtheroids" isolated by Dr. Esther Meyer and associates (**2**) of the University of Illinois Medical School. We consider, from their descriptions and from those of Weed and Baggenstross (**13**), the possible identity or close relationship to one or the other

of the species we studied. Our culture of *A. bovis* was ATTC *10084,* which has been found in our laboratories to be a typical strain. From its colonial and microscopic appearance we judge it to be in the S stage. We obtained 7 strains of *L. bifidus* from Norris. Our concept of this species is that of Norris *et al.* (**5**) and of the original investigators in the species, not that of some of the recent investigators, who would identify this species with *Lactobacillus acidophilus.*

Both *L. bifidus* and *A bovis* are known not to grow on the ordinary culture media of the medical mycologist and even in the more favorable bacterial media to grow only in the anaerobic portions well below the surface. We found this to be the case with all the cultures of the anaerobic diphtheroids as well as *L. bifidus* and *A. bovis.* Especially in thioglycollate broth (BBL) plus 0.2% agar this was apparent. Growth was seen only below the area where the E_H indicator, resazurin, showed that air had not penetrated. The other two media used were brain heart infusion broth (Difco) and the liquid "bifid medium" of Norris *et al.* (**5**) minus the B_{12} which Norris (personal communication) found to be unnecessary. Incubation was at 37° C for up to 1 week. Materials were examined after fixing and staining and also under wet mounts, but nigrosin "negative staining" without fixation gave most easily photographed results.

RESULTS

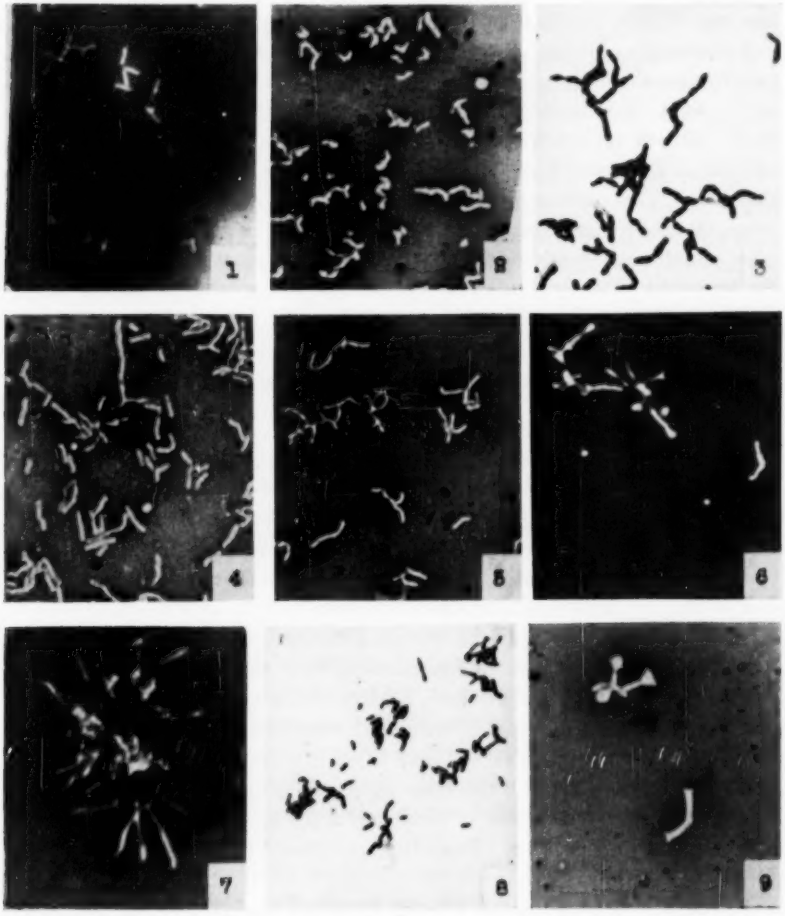
Although the examinations were made repeatedly over a period of 18 months the results can be briefly summarized. The wording here is taken from that of the report at the end of the experimentation. (Frank, H. A., M.S. thesis, State College of Washington, 1952.)

OBSERVATIONS OF THE TEST ORGANISMS GROWN IN TUBES OF THIOGLYCOLLATE MEDIUM

Actinomyces bovis: The colonies of growth formed by this organism are unmistakable. They are rather small, compact and lobulated. No growth is seen in the upper portion of the tube where oxygen penetration is indicated by the color change of the resazurin present in the medium. Growth is dispersed throughout the rest of the tube. Larger colonies tend to occur near the bottom of the tube. The colonies are very difficult to disrupt with an inoculating needle. Strains of this organism show a rather "pleomorphic" rod which stains irregularly. The morphology of the organism is not consistent. Variation in size and shape ranges from coccobacillary forms to clavate rods. Many straight, regular rods are also present. The occurrence of massive clumps of organ-

asms is very common. Some short chains can be discerned, and simple branching is common (FIG. 1).

Anaerobic diphtheroids: Strains of these organisms did not show any growth in the top 2 cm of the medium. Below this zone, they were characterized by heavy, mucoid, compact masses interspersed with smaller, more concentrated nodules to which a slight "comet-tail" was



FIGS. 1, 4, 7. *Actinomyces bovis.* 2, 5, 8. "Anaerobic diphtheroids"; 3, 6, 9. *Actinomyces bifidus.* 1, 2, 3 from thioglycollate broth; 4, 5, 6 from brain heart infusion broth; 7, 8, 9 from "bifid medium." 1, 2, 4, 5, 6, 7, 9, nigrosin without fixation; 3, 8, Gram stain. Magnification × 2000.

attached. The entire concentration has a slightly pinkish hue. Sometimes, growth was also present as a fine film covering the inner surface of the test tube. Often a heavy, pink sediment was obvious at the bottom of the tube. These organisms stain darkly and are rather chubby, short rods of a variety of morphological types. Most are coccobacillary and have tapered, but round ends. Chains are rather short and slightly twisted. Some V- and Y-shaped pairs are seen. Very simple branching and slightly budding projections are discernable (FIG. 2).

Lactobacillus bifidus: The strains of this species, similar to the other two species showed no growth in the zone at the top of the tube. With some slight variations in consistency, they all grew as slightly mucoid, fluffy, streaks resembling, often, a molar tooth with its roots intact. In one strain at least, 21–39, a heavy sediment was noted at the bottom of the tube. The most common type of organism seen was an extremely large, clavate form. Branching is prevalent. This is of a rather simple type, exhibiting in addition knob like projections at various points on the branches. Many V- and Y-shaped units were seen (FIG. 3).

OBSERVATIONS OF THE TEST ORGANISMS GROWN IN TUBES OF BRAIN-HEART INFUSION MEDIUM

A. bovis: This species grew characteristically. Clumps of large, compact, white, lobulated colonies resembling tiny cauliflowers were typical in this medium. A variety of morphological types were present. There were many uniform, straight rods, some coccoid forms and extremely pleomorphic ones with slightly bulbous ends. The occurrence of clumps is common. Short chains, and simple branching were frequent. Y- and V-shaped pairs were present (FIG. 4).

Anaerobic diphtheroids: All strains grew as heavy, mucoid, sedimentation films at the bottom of the tube. There was some difference between the strains in this group. Kesiter strain consisted of small rods with tapered, round ends. It exhibited some chains, but no branching. The other two strains, Viv *195* and Viv *1404*, consisted of many "pleomorphic" types. Mostly, the rods were small, almost coccoid in many cases, and of an assortment of sizes and shapes. Many chains of various lengths were present. Branching and budding were very common (FIG. 5).

L. bifidus: Growth of this species occurred mainly as a sparse fluffy film at the bottom of the medium. These cells stained uniformly. The knobby split ends of many rode are frequent. Other branched arrangements were seen, mainly Y- and V-shaped pairs (FIG. 6).

OBSERVATIONS OF THE TEST ORGANISMS GROWN IN TUBES OF "BIFID" MEDIUM

Since, in practically all instances, growth consisted of a filmy, spreading mass at the bottom of the tubes, description of the organisms in this medium will be restricted to microscopic characters.

A. bovis: This organism presented tangled masses of rather straight chains of organisms which stained irregularly. Branching was seen. Little "pleomorphism" was observed (FIG. 7).

Anaerobic diphtheroids: These strains were comprised, almost without exception, of small, regular, rather fat rods which stained quite easily. There were chains of almost coccoid rods and infrequently simple branching cells (FIG. 8).

L. bifidus: The tremendous assortment of shapes and sizes evoked by this medium is startling. The rods are rather large and vary in shape from coccoid to medium-sized bacilli. There is a marked tapering at both ends of the cell. The Y-shaped, or "bifid" type, is common. Small chains occur occasionally. The split, knobby ends of many organisms were prominent (FIG. 9).

SUMMARY AND CONCLUSIONS

Lactobacillus bifidus is sufficiently unlike *Actinomyces bovis* in morphology (and in fermentation characters, not reported here) clearly to be another species. The anaerobic character and the definite branching of the cells are sufficient to remove the former species from the genus *Lactobacillus* and to designate it *Actinomyces bifidus* (Tissier) Nannizzi in Pollacci, Trat. Micopat. Umana **4**: 13. 1934. These studies confirm the general conclusions of Negroni and Fischer (**4**).

We have found no described species to which we can assign the "anaerobic diphtheroids." Meyer is currently studying them with a view to classification. We believe that they belong to the genus *Actinomyces* in the restricted sense of Waksman and Henrici. The strictly anaerobic character and the rudimentary branching mycelium set them off from the genus *Corynebacterium*. They are not to be identified with either *Actinomyces bovis* or *Actinomyces bifidus* but seem to be close to the former.

Finally the junior author wishes to repeat the substance of a statement he published in 1947 (**10**) to which not a few microbiologists, both bacteriologists and mycologists, have taken exception in private oral or written communications. Some of the actinomycetes (e.g. the genus *Streptomyces*) are more closely related to the true fungi than they are

to certain of the other actinomycetes such as *Actinomyces bovis*, which in turn is more closely related to the true bacteria than to such actinomycetes as species of the genus *Streptomyces*. The present study tends to confirm the validity of the statement. That most bacteriologists have, until very recent times, classified all the actinomycetes in one genus, and at present in a single order of the Schizomycetes, is not necessarily a valid reason for maintaining this monophyletic concept. However, if

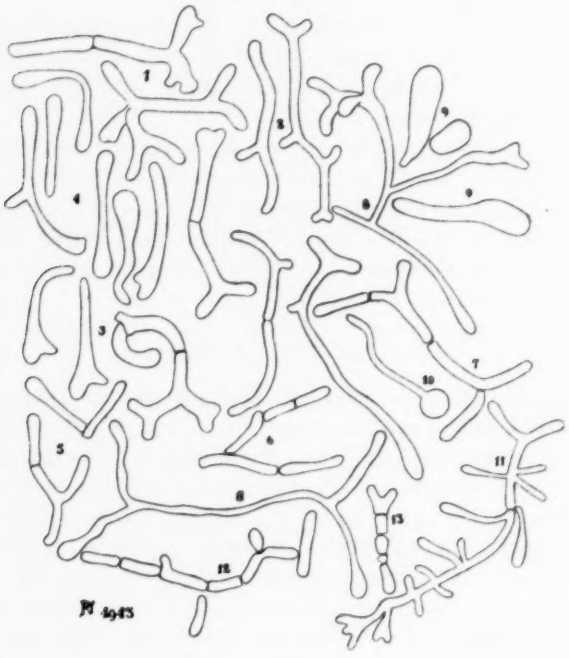


FIG. 10. Camera lucida drawings by Negroni and Fischer (**4**) of *Actinomyces bifidus*. Reproduced by permission.

the actionomycetes, in whole or in part, are classified with the true fungi, the persons making such transfers should be familiar with the type species of the genus *Actinomyces*, namely *Actinomyces bovis*. Clearly most of those who insist on the fungal nature of all the actinomycetes are not familiar with the type species of *Actinomyces*.

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CELLULAR MECHANICS, TAXONOMY AND EVOLUTION IN THE UREDINALES AND USTILAGINALES [1]

D. B. O. SAVILE [2]

(WITH 26 FIGURES)

The following notes are largely the incidental byproducts of cytological and taxonomic work, which have accumulated in my notes or in the back of my mind for a number of years. They are not intended as a complete or connected discussion of cellular mechanics in the rusts and smuts, some phases of which, such as basidiospore discharge, have been treated extensively by several authors. Most of the aspects discussed below have received little attention. An understanding of some of these topics may be of service in the interpretation of observations and in the development of rational classifications and phylogenetic schemes. Some of the phenomena were not fully appreciated until I recently restudied D'Arcy Wentworth Thompson's (**25**) *On Growth and Form*.

On Growth and Form has perhaps not received adequate attention from mycologists; at least it is seldom quoted by them. Yet many of the principles that the author discusses are applicable to the fungi, although most of his illustrative examples are drawn from the animal world. This book may fairly be regarded as the distilled wisdom of one of the world's intellectual giants; and every mycologist—not least the taxonomist—could well reread it every few years just to keep his feet on the ground. To avoid quoting repeatedly from it, and adding needlessly to the length of this paper, the reader is referred to the book for supporting details in some of the following paragraphs.

The illustrations of spores and spore walls are from original cytological preparations or whole mounts of spores in lactophenol.

BASIDIOSPORE FORMATION AND SURFACE TENSION

The basidiospore initial on a sterigma is a sphere at the end of an unduloid. The form of the combined structure is precisely that of a

[1] Contribution No. 1361 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Senior Mycologist.

droplet parting from the body of a liquid. See FIG. 3 and Edgerton's photographs reproduced in frontispiece and facing p. 390 of Thompson (**25**) for comparison with FIGS. 1 and 2. In the splash, a droplet is forced away by the kinetic energy of the moving liquid. In the rust, the droplet (at this stage there is no wall in the accepted sense) is abstricted by an unidentified repulsive force. In each instance surface tension is the force governing the form. It is not necessary to assume genetic relationship wherever such a structure is found in the fungi. It may, for example, be closely duplicated in the conidiophore of some Phomales.

Departure of the abstricted spore from the spherical form demands the continuing consumption of energy until a firm wall has been laid down. A substantial departure from the sphere may often be functional and the result of evolutionary development. In the absence of conflicting evidence we may tentatively regard the spherical basidiospore as more primitive than the reniform or ellipsoid. Spherical basidiospores are general in many genera of Melampsoraceae, but the reniform or ellipsoid shapes are usual in Pucciniaceae.

TELIOSPORE EVOLUTION IN PUCCINIA AND UROMYCES

The primitive condition in *Puccinia* and *Uromyces* is that in which the spores are densely compacted in a firm palisade, and have firm, often short pedicels. The spore wall is smooth, as is to be expected since the spores germinate *in situ*. In fact in many, at least, of these species detached teliospores cannot be made to germinate. The germpores are apical and at the septum, the positions that bring the basidia free from an enveloping film of water with the least growth. The spore wall is usually strongly thickened at the apex, perhaps partly as a protection from the weather, but probably mainly as a protection against small molluscs or insects (notably gall-midge larvae). Simple or branched apical appendages, such as we see in *P. coronata*, *P. praegracilis*, *P. volkartiana* and, especially, in the aptly named *P. rangiferina*, carry this protective device one stage further, forming a sort of brush pile over the sorus. Thus any animal must eat its way through much nearly valuless wall substance before reaching the nutritive protoplasm.[3]

It is obviously advantageous if the teliospore can play a distributive rôle as well as its normal sexual and overwintering one, especially in microcyclic species or at high altitudes and high latitudes where the uredinial generations are greatly reduced. In the microcyclic species,

[3] Yarwood *et al.* (**27**) have recently indicated that in *Uromyces fabae* the high nutritive value of the rust protoplasm is due to its large pantothenic acid content.

if the teliospores remain attached, spread is limited to the quite small effective range of the basidiospores, except for accidental transfer with the host plant, e.g. on ice floes or by wind or animal action. Such species may be stopped indefinitely by a narrow sea barrier, a mountain range or a dry valley unsuited to the host plants.

In many species of *Puccinia* and *Uromyces* a distributive function has been achieved for the teliospores by the pedicel abscissing, not right at the spore as in the urediniospore (FIG. 11) but a few micra away from the spore (FIG. 4). Once the pedicel becomes deciduous there is no longer any appreciable advantage in the spore wall being apically thickened, for the spores no longer form a firm and regular palisade but are piled loosely on the sorus as they are pushed up from below by successively developing spores. Some spores are detached by this upthrust; others are detached by wind action, insects feeding on the sori, or by other means. The decreased protection from mycetophilous animals is offset by greater mobility and the ability to produce successive crops of spores from the same sorus.

With increasing fragility of the pedicels there is a decreasing chance of teliospores germinating *in situ*. More and more they will lie detached at the surface of a drop or film of water, where they will become oriented with the long axis more or less parallel with the water surface. Consequently the advantage, in *Puccinia*, of having the pores strictly apical and at the septum is lost. There does not seem to be any great advantage in the lower pore being slightly depressed, although increased separation of the basidia may increase the chance of all basidiospores being successfully discharged; but there is probably some advantage in its being nearly basal, for then it is close to the water surface however the spore rotates.

In the firm-pedicelled, immobile teliospore, wall sculpturing, as distinct from apical appendages, would be completely functionless; but in a deciduous-pedicelled species scupturing serves both to give the spore added buoyancy when it is air-borne and to increase its chances of being transported by insects or other small animals.

In consequence of these facts it is not surprising that, correlated with the evolution of the deciduous pedicel, we find a strong tendency toward walls of uniform thickness, depressed germ-pores, and variously sculptured walls.

Although the division of *Puccinia* by Arthur (1) into two sections, *Eupuccinia* for the firm-pedicelled and *Bullaria* for the fragile-pedicelled species, has some practical advantages, it has become increasingly clear that the division is not a natural one; for the transition from firm to

deciduous pedicels has taken place repeatedly in both *Puccinia* and *Uromyces*, as the study of several closely related groups of species shows us. A few examples will suffice.

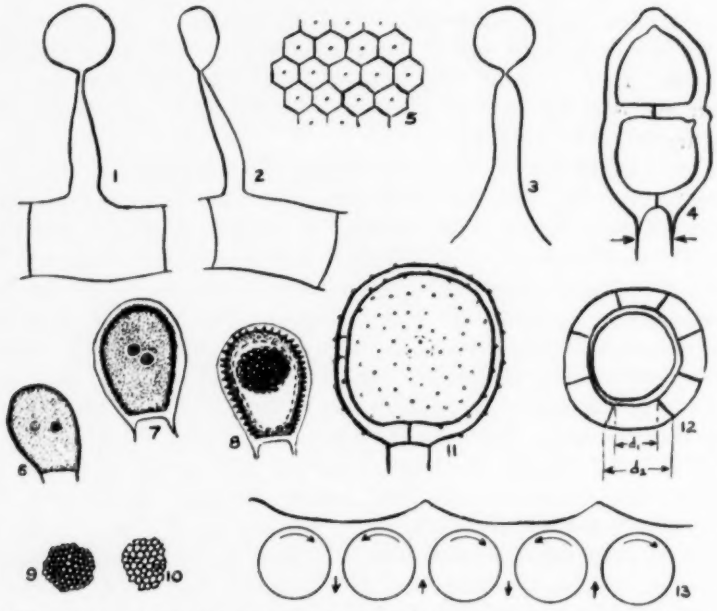
There is a group of *Uromyces* spp. attacking Plumbaginaceae (**23**), all clearly related as shown by the morphology of the peridial cells and the closely set, broadly conic warts of the urediniospors. In *U. limonii*, *U. limonii-carolinianae* and *U. statices-sinensis* the teliospore pedicels are firm. In *U. armeriae* the pedicels are delicate and more or less deciduous, especially in ssp. *pacificus*. Finally in *U. acantholimonis* they are definitely deciduous.

In the microcyclic species of *Puccinia* on Saxifragaceae, recently monographed (**21**), we can trace an almost unbroken series of taxa from several Asiatic species with strongly to weakly persistent pedicels, through several varieties of *P. heucherae* with the pedicels ranging from weakly and spasmodically persistent to fully deciduous, to the several varieties of *P. pazschkei* all with deciduous pedicels. This sequence is accompanied by the progression from smooth, through finely striate and strongly striate to coarsely rugose walls, and often by some depression of the lower pore. It is also accompanied by a progression from the older to the more modern genera and species of host plants.

For a third example we may consider a large group of species of *Puccinia* and *Uromyces*, attacking various unrelated plants, both dicotyledonous and monocotyledonous. The kinship of these species is shown by their common possession of several correlated urediniospore characters. These characters are: urediniospores appreciably flattened; germ-spores two, superequatorial and on the flattened faces; and a circular area, below each pore and reaching nearly to the base of the spore, devoid of echinulations. This last character was noted in various species long ago by Klebahn (**14**), but has been overlooked by most authors, partly, no doubt, because such mounting fluids as lactophenol clear the spores so thoroughly that the delicate spines are often difficult to see. Examples include *Puccinia extensicola*, *Uromyces perigynius*, *U. silphii*, *P. obscura*, *P. aletridis*, *P. cypripedii*, *P. proserpinacae*, *P. punctata*, *P. acetosae*, *P. hieracii*, *P. harknessii* and *P. chondrillina*. All these characters vary somewhat in degree from species to species, and even between races or varieties within a species; each thus seems to be controlled by several genes. It is almost unbelievable that such a combination of characters has arisen repeatedly, especially as they seem to have no particular survival value. Yet species having all these characters occur in both the firm-pedicelled and deciduous-pedicelled groups and, within each group, on a variety of hosts. It must be assumed that

even in this single section of *Puccinia* (including correlated species of *Uromyces*) the transition from firm to deciduous pedicels has occurred several times.

There is a smaller, related series in which the pores are disposed



FIGS. 1–13. 1. Basidiospore formation in *Melampsora epitea*. 2. Basidiospore formation in *Uromyces lespedezae-procumbentis*. 3. Late phase of splash in milk, showing droplet separating from columnar jet; after plate facing p. 390 of *On Growth and Form*. 4. Teliospore of advanced species of *Puccinia;* arrows indicate zone of weakness in pedicel. 5. Pattern of regular hexagons; emphasis of cell margins reveals hexagon pattern with spines, if any, at nodes of reticulum; emphasis of cell centers, such as reversal of circulation would cause, would give protuberances in a pattern of equilateral triangles. 6. Early stage in formation of urediniospore of *P. antirrhini*. 7. Slightly later stage showing spine pattern imprinted into gelatinized primary wall. 8. Slightly later than FIG. 7, with bolder pattern and primary wall starting to shrink, equatorial and partial surface view. 9. Upper surface of same spore with slightly higher focus, showing crowded circles tending to reticulum. 10. Even higher focus showing incipient spine at each node of reticulum. 11. Mature urediniospore. 12. Section through smut spore covered with high meshes; diameter of mesh at base (d_1) is much less than at top (d_2). 13. Section through strip-thermal type of circulation; curved arrows show rotation of cylindrical systems; vertical arrows show up- and down-drafts; if such a circulation exists close to the surface of a young spore the membrane will be thrown into folds as shown at top of diagram.

equatorially on the flattened faces of the spores and the smooth area surrounds them. *Puccinia helianthi*, the familiar rust of sunflowers, is one such species. In the races on most species of *Helianthus* the teliospores are firm-pedicelled, but in one well marked segregate, which certainly merits varietal rank, the pedicels are very delicate and might be termed semi-deciduous. In the related *P. helianthellae*, on *Helianthella* spp., the pedicels are fully deciduous. In *P. balsamorrhizae*, on the related genus *Balsamorrhiza*, the pedicels are fully deciduous and the teliospores are finely verrucose.

The reader is entitled to ask how it is that, if the *Bullaria* section of *Puccinia* is not natural, the teliospore pedicels of the various species comprising it almost invariably break off at the same point a few micra from the spore. This single point greatly delayed my full acceptance of the multiple origin of these rusts. The explanation is purely mechanical and is almost absurdly simple. Although it is not obvious in unfixed spore mounts, in fixed and stained thin sections we find that at the septum between spore and pedicle the pedicel wall is usually fully as thick as the spore wall. The thickening tapers to a minimum in a short distance, as shown in FIG. 4, whether the pedicel is persistent or deciduous. The remainder of the pedicel has approximately this minimum thickness. Obviously a delicate pedicel must break at or below the point indicated by the arrows. Occasionally it will break irregularly at some point lower down, but usually we see a relatively clean break at the bottom of the tapered portion. As the mechanical engineer well knows, any such discontinuous surface is a point of weakness. Thus sharp angles, particularly interior angles, are always avoided in highly stressed structures that are subject to vibration; for minute cracks originate at them and cause fractures.[4] Repeated stresses on the spore, such as might be caused by vibration of the plant, wind action on the spores or the upthrust of young spores in the sorus, will generally cause the pedicel to fracture at the base of the taper even if the rest of the pedicel wall is equally thin. Thus we have a good example of a physical relationship simulating a biological relationship. Once we realize that the relationship is physical, the multiple origin of the deciduous-pedicelled species is obvious.

It may be noted in passing that the wall of the urediniospore pedicel (FIG. 11) tapers much more abruptly than that of the teliospore. Ac-

[4] The argued relative merits of the inexpensive American truncated-vee thread and the stronger, rounded British Whitworth thread delayed the adoption of an international standard screw thread for years, and cost the western allies many millions of dollars.

cordingly it not only fractures much closer to the spore, but the discontinuity is so pronounced that the break invariably occurs cleanly at this point.

EFFECT OF PRESSURE IN THE SORUS UPON TELIOSPORE SHAPE

Speaking in very general terms, we may say that the firm-pedicelled species of *Puccinia* tend to have long, slender spores and that the deciduous species tend to have short, broadly rounded spores. To a large extent this change in shape may be one of the correlated genetic changes accompanying the evolutionary sequence from one type to the other; but to some extent the change is probably mechanically controlled. In a firm telium, such as those of most grass or sedge rusts, the spores are tightly packed in a palisade-like layer. Once the spaces between them are filled, spores in the middle of the sorus can make no further growth except upward. In many such rusts we find the spores at the edge of the sorus to be shorter and broader than those in the middle. In comparing specimens, spores of each should be taken from both the edge and the middle of the sorus. In a species with fully deciduous pedicels the spores tend to break loose and be pushed upward successively as new ones form, and probably they are not usually subject to much pressure.

The rust on *Achillea ptarmica* was considered to have broader teliospores than those of *Puccinia millefolii* on *A. millefolium*, and was described as *P. ptarmicae*. Actually the range of spore widths is the same, but wide spores are commoner on *A. ptarmica* than on *A. millefolium*. The difference appears to be due merely to mechanical pressure from the host tissues being less in the former host than in the later; for the slight difference has been seen even when the rust spread from one host to the other.

SPORE WALL ORNAMENTATION

The form, function, and means of formation of spore wall ornamentation is an absorbing field, and one that has been inadequately investigated. All spore markings may be assumed to increase the buoyancy of air-borne spores, but it is by no means certain that this is always their main function. In cupulate aecia, for example, the aeciospores are finely verrucose and, as shown in a later section, the prime function of these markings seems to be to aid in spore discharge. In *Cintractia aspera, C. fischeri,* and especially *C. subinclusa* we have close, high warts

that tend to trap air bubbles and ensure that the spores float indefinitely on water, an important provision in these smuts, whose hosts are generally aquatic (**18**, **22**).

In finely verrucose spores the production of the wall markings is very difficult to follow, but the mechanism has been worked out for the echinulate urediniospores of *Puccinia* and *Uromyces* with suitable staining methods, although the basic forces involved are a matter for conjecture. When the urediniospore of *Puccinia* or *Uromyces* is still much less than full size the primary, and only wall becomes gelatinized, swelling to several times its normal thickness. Through the violent activity of the cytoplasm a system of conical pits is formed on the inside of the gelatinized wall, which thus becomes a matrix (FIGS. 6, 7). The secondary wall, which provides almost all the thickness of the mature wall, is now laid down against this matrix in a manner analagous to centrifugal casting. When the pits have been filled and a substantial layer of material has been laid down behind them, the gelatinous layer shrinks down to its original thickness, ca 0.1–0.2 μ, taking the shape of the spines as it does so. The analogy with the casting process is thus incomplete, for the matrix is not removed from the finished product. Finally the growth of the spore is completed, presumably with the addition of wall material by intussusception (FIG. 11).

If a spore in the matrix stage is examined in surface view it will be seen that the pits in which the echinulations are to be laid down form a pattern approaching that of regular hexagons (FIGS. 8–10). There are two related patterns that result from packed circles: the hexagon pattern is evident when the boundaries are seen; but the equilateral triangle pattern shows up when the centers of the circles are emphasized (FIG. 5). In the mature spore the original pattern suffers distortion in the somewhat uneven growth of the wall, and is usually obscure. When the pattern is first laid down it is much more regular, but it is so fine that good cytological preparations and high resolution are needed for its study. In the course of cytological studies (**16**) it was elucidated in some detail for *Puccinia antirrhini*, and enough was seen to indicate that the same sequence was followed in *P. hieracii* and *Uromyces fabae*.

As FIG. 8 illustrates, focussing low in the surface of the young spore reveals a pattern of clear circles (matrix) with densely stained cytoplasm between. As the focus is raised (FIG. 9) the intervening reticulum becomes so fine that it is difficult to see clearly even with the highest useful magnification at N.A. 1.30. However, it has been seen in *P. antirrhini* that the circles enlarge to become crowded into polygons (usually hexagons, FIG. 10); and that finally we have no reticulum but

only a delicately stained dot (the young spine) above each node of the reticulum. This point is of some importance because it illustrates the essential unity of the mechanisms forming echinulate and reticulate spore patterns. The reticulate pattern does not show in the mature urediniospore, but it is noteworthy that it can just be detected sometimes in the aeciospores of *Melampsora epitea* (*M. bigelowii*) on *Larix*.

Both reticulate and echinulate spores are well known in the smut fungi, and in the beautifully marked spores of *Ustilago parlatorei* the patterns are combined. The walls are covered by a high reticulum with a distinct spine at every node (**20**, p. 672).

No mater how regular the meshes on a reticulate smut spore appear to be, examination shows that a perfect net of hexagons never exists; there are always 4-, 5-, or 7-sided figures interspersed. The search for a perfect net need not detain us, for, as D'Arcy Thompson points out, Euler proved some two centuries ago that no system of hexagons can enclose space. In designating the diameters of the meshes one author was at pains to specify diameters across flats or across angles, although no such distinction is possible when some of the polygons have an odd number of sides. In small spores that bear large, high meshes there is a much more important precaution for us to take. The opposite walls of such meshes are at a substantial angle to each other (FIG. 12), and the higher the meshes the more the diameter changes from top to bottom. In such spores it is advisable to state at what level the mesh diameters are measured in recording the range of variation.

We do not know the precise forces in the outer part of the cytoplasm that form these wall patterns, and perhaps we never shall. The activity of the surface layer is an indication of instability, and we may accordingly be sure that differences in surface tension and electric potential occur; but with our present knowledge it does not seem possible to tell whether potential difference initiates unequal surface tension or *vice versa*. There may also be other forces, chemical and physical, involved. What we do know is that the system of circular "cells" at the surface of the cytoplasm, whether crowded into polygons or not, is the result of instability. As D'Arcy Thompson has shown (**25**, ch. 7), there are numerous biological and physical examples of this phenomenon, both microscopic and macroscopic. As he has pointed out, James Thompson many years ago recorded a polygonal or tessellated pattern on a tub of suds. The slowly cooling suds chill faster on the exposed upper surface than in the body of the liquid; and the surface layer is consequently denser than that below, causing the mass to be unstable. In the absence of appreciable agitation, instability of this sort is cor-

rected by the fluid welling upward in cylindrical columns and settling in the boundary zone (reticulum) between them or *vice versa*. It has been shown in cloud-chamber experiments that the system may change abruptly from one direction of flow to the other. The full explanation is too complicated for us, but the interested reader will find some discussion and references in Humphreys (**11**, ch. 16).

Exactly the same phenomenon is easily seen on a vastly greater scale in cirrostratus and less clearly in altostratus cloud sheets; and here the sequence is constant and the reversal may, with patience, be quite frequently observed without any laboratory equipment, although only fragments of the process seem to have become incorporated in text books on meteorology.[5] The process is evidently initiated by subsidence of the cloud sheet, during which it is warmed dynamically (i.e. by compression). The top of the sheet is warmed less than the bottom, and the sheet consequently becomes unstable. At first clear holes appear in the sheet, indicating columnar downdrafts with consequent warming of the contained air and evaporation of the ice crystals or water droplets. This process usually continues until parts, at least, of the sheet are reduced to a mere reticulum, such as was illustrated by Clarke (**6**, pl. 12 b) and aptly termed change cirrus although its origin and significance do not seem to have been fully appreciated. If the cloud does not now evaporate entirely, through further subsidence, we get a sudden reversal of the pattern, with columnar updrafts and intervening subsidence giving the familiar rounded cloudlets of cirrocucumulus.

This process in cloud transformation is important to us because it throws light on various modifications of the process that we have described in spores. For example, if we watch the same process at the edge of a lenticular patch of altostratus, we see that the clear columns are much smaller in the thin peripheral part than in the thicker cloud a little farther from the edge. Thus we see that a minor change in the conditions may greatly affect the scale of the pattern. We need not be dismayed at minor inequalities in the pattern on a spore wall: rather we should be astonished that any sort of regularity can ever occur.

In discussing our cloud analogy we dealt only with the simple case where there is no difference in velocity between the upper and lower surfaces of the cloud; a relatively uncommon case, since the sheet is ordinarily at the interface between two air masses. The shearing force that results from such unequal velocities produces effects that are too

[5] Probably more information is available in original publications than in the textbooks upon which the layman must largely depend. This discussion is based largely on fourteen years' personal observations.

complex for us to follow here completely, but the results are readily observed in the cloud sheet. With even a relatively small difference in velocity the rounded cloudlets become horizontally elongated, giving the familiar mackerel sky with a pattern much like that seen on rippled sand after the tide retreats. Such an effect occasionally occurs in rust teliospores, e.g. *Uromyces spragueae*, and in smut spores, e.g. in the markedly elongated meshes on the spores of *Cintractia pachyderma* and *Tilletia elymi* illustrated by Fischer (**8**).

The extreme case of shearing in the atmosphere is illustrated in what the sailplane pilot terms a strip-thermal. Under certain conditions of temperature and wind velocity the columnar thermals rising from the sea, or a uniform land surface, are transformed into horizontal rotating columns, lying in the direction of the wind and each rotating in the opposite sense from its neighbours as if all were geared together (FIG. 13). Thus at the junctions we have alternate updrafts and downdrafts. The updrafts are often strong enough to permit a gull (or sailplane pilot) to soar directly upwind. Here we have a clue to the development of striate spore walls such as are found in *Ustilago piperii* or *Puccinia heucherae* var. *saxifragae*. All that is required is a pronounced shearing force superimposed upon a uniformly unstable surface film. In spores such as those of *P. heucherae* var *saxifragae* the striae usually run nearly symmetrically from end to end (FIG. 14). They may be looked upon as the visible indications of a field of force between two poles, analagous to the pattern of iron filings between the poles of a magnet. In the striate spore the forces involved must cause a unidirectional streaming from one pole to the other at or very near the surface of the cytoplasm, with a deep return flow. In this early stage of its development the cytoplasm has no large vacuoles and probably has not yet built up the droplets of fatty material that are conspicuous in the mature spore. Only the nuclei are present to offer appreciable resistance to the return flow and they are still in the small, unexpanded form that immediately follows the division into spore and pedicel.

Although we do not know just what forces induce this shearing flow, their location is often clear. In some collections of *Puccinia heucherae* var. *saxifragae* we find appreciable numbers of irregularly shaped spores (FIGS. 15, 16). From such spores we see that one pole is at the pedicel attachment, and the other at the papilla over the germ-pore that is furthest from the pedicel, usually, but not invariably the pore of the upper cell. The other papilla usually plays little part; but it may act as a secondary pole, especially if both pores are nearly equidistant from

the pedicel, causing some of the striae to be deflected from their usual smooth, nearly geodetic [6] curves.

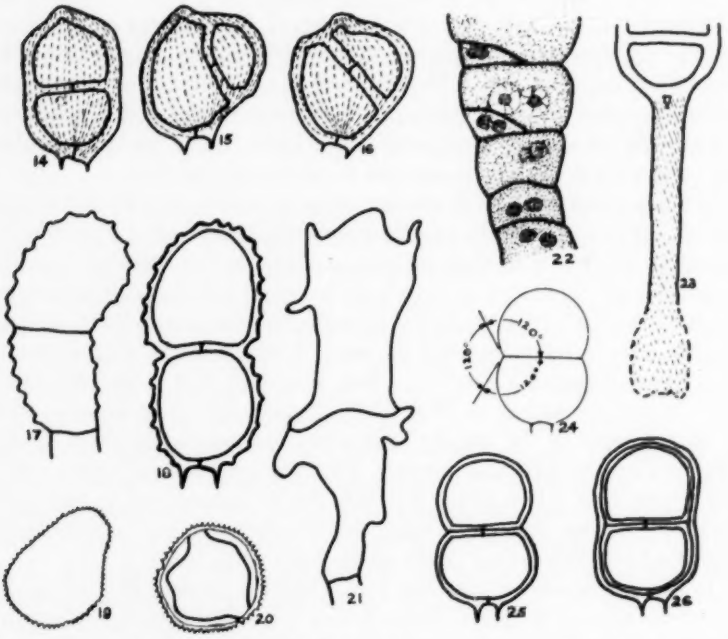
It is important to know how widespread is the mechanism of laying down spore markings in a matrix. I have not definitely followed the process through in the smut fungi, but in various reticulate-spored species of *Tilletia* and *Ustilago* one sees a scattering of cells with thick, smooth, subhyaline walls, which may represent spores in which development was checked after the swelling of the primary wall has occurred. It is interesting to note that Fischer and Hirschhorn (**9**) found that, by mounting in Shear's fluid, a gelatinous outer sheath can be demonstrated on the spores of some of these species. It is suspected that this sheath is the primary wall that shrank down after the reticulum was completed but can be re-swollen in this particular fluid.

In finely verrucose spores the sculpturing process is more difficult to follow, but it may often be identical with that described. It is difficult, in fact, to think of a mechanism that is not at most a modification of it. In cytological studies of *Tranzschelia fusca*, in which the tubercles are relatively high and not so closely spaced as to hinder examination, cytoplasmic activity seems to form the warts in the primary wall without it becoming gelatinized (FIG. 17). The secondary wall is then laid down into this matrix as before (FIG. 18). Exactly the same process seems to be employed in the closely echinulate-verrucose aeciospores of *Melampsora epitea* (*M. bigelowii*). The ungelatinized primary wall is thrown up into points (FIG. 19) and the secondary wall is laid down upon it. In this instance a more or less distinct third layer is finally deposited everywhere but at the germ-pores (FIG. 20).

In the teliospores of *Puccinia podophylli* an entirely distinct process of ornamentation is seen. When I first examined cytological preparations of this species I was horrified to find what seemed to be the most violent imaginable shrinkage, during processing, of all immature spores beyond a certain stage. It was soon apparent that the preparations were undamaged and that the collapse was part of the process by which the conspicuous spines of the mature spores are formed. FIG. 21 shows an immature spore in which spine formation has started in the lower

[6] Geodetic curves are briefly treated in chapter 10 of *On Growth and Form*. They are probably incidental in the present example, but represent an important constructional principal, well represented in vascular plants. But for high costs the principle would be more widely used in engineering. This construction resists torsion by distributing stresses among various members. The classic engineering example was the Wellington bomber, which used to return from action despite fantastic amounts of wing or fuselage having been shot away. The principle should be watched for wherever spiral or helical structures occur among cryptogams.

cell and is well advanced in the upper. While the spores are still quite small, and the wall not appreciably thickened, a sudden contraction of the protoplasm throws the wall into irregular folds. The process can be seen under the microscope essentially in only two dimensions, but it is actually three-dimensional. If, as the sections usually suggest, each crest were formed between two wrinkles, the wall would eventually be



FIGS. 14–26. 14. Typical spore of *P. heucherae* var. *saxifragae;* poles at pore of upper cell and at pedicel attachment. 15. Atypical spore of same rust; pole at pore of lower cell. 16. Atypical spore of same rust; primary pole at pore of upper cell; secondary pole at pore of lower cell. 17. Young teliospore of *Tranzschelia fusca;* pattern imprinted on ungelatinized primary wall. 18. Nearly mature spore of *T. fusca.* 19. Immature aeciospore of *Melampsora epitea* on *Larix;* pattern imprinted on ungelatinized primary wall. 20. Mature aeciospore of *M. epitea.* 21. Immature teliospore of *Puccinia podophylli;* formation of spines by shrinkage process is more advanced in upper cell than in lower. 22. Aeciospore column in *Uromyces fabae,* showing invagination of spores by intercalary cells. 23. Spiral structure in pedicel of *Phragmidium americanum,* which causes spore to rotate about long axis during uptake or loss of water. 24. Diagram of *Puccinia* teliospore, showing form due to surface tension uncomplicated by other forces. 25. Teliospore in which all thickening occurs after septum is laid down. 26. Teliospore in which half of thickening occurs before formation of septum.

decorated with ridges. The production of spines requires that three or more areas of collapse center around a point. The situation is analogous to that in mountain glaciation where plucking of rock from the walls of two adjacent valleys eventually yields a razor-back between them, whereas the action of three or more cirques about a mountain gives rise to a matterhorn peak. In *P. podophylli* some wall thickening occurs as soon as the spines are formed. Thus the sides of the spine are locked together and it cannot become unfolded when growth of the spore is resumed. Lamb (**15**) investigated *P. prostii,* which has very similar teliospores, and found that the same pronounced folding occurs in the young spores of that species. It is safe to conclude that the process is identical with that in *P. podophylli.* It seems probable that much the same process occurs in *Nyssopsora,* where it presumably evolved independently. Parenthetically it may be noted that the main function of these large spines must be to aid in dispersal of the spores by mites, insects and perhaps other animals.

This process is essentially one of shrinkage, followed, in this case, by renewed growth. Shrinkage patterns are common in various groups of plants and animals, and Thompson (**25**, ch. 5) gives various examples. Among the examples most familiar to bontanists are wrinkled, reticulate, or grooved seed coats, the last being well exemplified when shrinkage occurs in seeds with flattened facets such as those of *Euphorbia glyptosperma.* Shrinkage patterns may be relatively common in the fungi. The shallowly undulating walls of the sporangia of some Phycomycetes suggest this mechanism. The strikingly angular spore forms of some species of *Cintractia,* e.g. *C. caricis, C. irregularis,* and *C. sclerotiformis,* appear to be due to shrinkage. They are certainly not due to mutual compression in the sorus, as a correspondent has recently suggested. Compression patterns, which we shall discuss below, are very different in appearance. In finely and shallowly verrucose spores it is difficult to tell what process is involved. The pattern may sometimes be due to shrinkage, or even a combination of methods, but the formation of a matrix by cytoplasmic activity seems to be the more important process.

One other curious method of achieving a rough spore coat has been described by Thirumalachar and Narasimhan (**24**) for the smut *Zundelula fimbristylidis,* although not strictly a sculpturing process. The spores, invested in a pseudoparenchyma, are scattered through a spore ball. When the spore ball breaks up the remnants of the pseudoparenchyma adhere to the spores in something approaching an irregular reticulum.

One lesson, in particular, emerges from the study of spore decoration: we cannot safely base relationship solely on wall pattern. In the rusts and smuts most of the wall patterns seen have developed repeatedly. To look at it differently, relatively few alternative types of development are open to any evolutionary group; and, given sufficient time, every group is likely to try out most if not all alternatives. The purple-spored species of *Ustilago*, a natural group largely confined to centrospermous hosts (**20**), illustrate this point. Among them we find species with verrucose, striate, reticulate, and reticulate-spinulose walls. In the species of *Cintractia* attacking Caricoideae the evolutionary pattern is unusually clear (**18**, **22**); and it is evident that verrucose spores have developed at least three times in this group during evolution from a smooth-spored form close to *C. carpophila*. It is also clear that angular spores have developed at least twice, once in the *C. caricis* complex attacking *Carex* subgen. *Eucarex* and once in *C. sclerotiformis* attacking *Uncinia*. Many other examples could be cited.

The foregoing argument is not a condemnation of spore morphology as an indication of relationship. All that is intended is a warning against implicit faith in a single character when that character may quite as easily indicate mathematical as biological relationship. In this respect I do not go nearly as far as did Thompson (**25**, ch. 5) in discussing the *Foraminifera*. He would explain the variously patterned shells solely on a mathematical basis, disavowing the contention of various morphologists that, within each genus, the smooth forms are ancient and the decorated forms are derived from them. The cases are, of course, not too closely analagous. The oceanic Foraminifera are subject to entirely different conditions from those to which aerially disseminated fungus spores are exposed. Perhaps decoration serves no useful purpose in the Foraminifera; but in the rusts and smuts (as in many other fungi) it is clearly an aid to dispersal, and is often, at least, an advanced character. As we have seen in our discussion of teliospore evolution, spore decoration has repeatedly followed the advance from firm to deciduous pedicels.[7]

[7] If any reader is inclined to the counter argument that pedicels evolved from deciduous to firm, and spore decoration has consequently been eliminated as useless, it may be noted that there is an enormous mass of data to the contrary in the form of correlations with life-cycle reduction and host relationship at levels ranging from order to species. To accept this opposing view would necessitate a complete inversion of the classifications of both the rusts and the flowering plants. Moreover, it would be odd if many derived species did not retain even a trace of the ancestral sculpturing after becoming firm-pedicelled.

AECIOSPORE DISCHARGE

The forcible discharge of spores from the cupulate aecium is a familiar phenomenon. Although the general principle involved is quite clear, some details have not been fully appreciated. The discharge depends essentially on turgor building up until it overcomes the restraint imposed by friction between adjacent spores and between spore and peridium. The friction is supplied by the strongly verrucose or toothed inner walls of the peridial cells (the outer walls are nearly smooth) and the closely but shallowly verrucose spore walls. Obviously the sparsely echinulate wall commonly possessed by urediniospores would be poorly suited to this mechanism, since 90% or more of the surface is actually smooth. Large cog-like warts, such as occur in the aeciospores of *Chrysomyxa*, which are not forcibly discharged, would reduce the efficiency of the spore as a missile, increasing drag out of all proportion to mass (and hence momentum). The long teeth of the peridial cells probably assist by flexing under pressure, and relaxing, to maintain close contact between spores, when discharge of a spore causes a lateral shifting of the uppermost layer of spores.

If the spores were thrust out simply by the upthrust of the developing spores deep in the aecium, the discharge would not be particularly violent, since there would be no sudden movement over an appreciable distance. One might visualize a sheet of spores spanning the aecium in a hollow curve, locked together, and finally the sheet being evaginated and shattered by mounting pressure. But this is not what occurs: we see the spores shot out one by one. What we need is for each spore to be invaginated from below; then the wall will suddenly evaginate as pressure overcomes friction and the spore will be flicked out. Dodge (**7**) many years ago noticed plugs in the germ-pores of the aeciospores of *Gymnosporangium ellisii* and suggested that they serve as fulcra to facilitate discharge. Dodge used a tennis ball and a marble as a model. If the tennis ball is pressed down upon a table and released abruptly it barely leaves the table; but if it is pressed down over the marble and released it leaps high into the air. The model works beautifully and is probably the key to the phenomenon of aeciospore discharge, but unfortunately the pore plugs do not seem to play the part of the marble. When I first noticed these plugs to be well developed in the aecia of *Puccinia caricis*, and, in searching for information about them, came upon Dodge's explanation, I tested the horizontal distance to which the spores were ejected from the aecia of various rusts with and without pore plugs. Some of the data are shown in TABLE I. It soon became evident that the presence or absence of the plugs had little or no effect

on the distance of discharge. The results were very variable for a given species and obviously depended largely on the turgor of the tissue. It was necessary to keep the pieces of leaf well flooded with water to obtain any spore discharge. It is notable that aecia of *P. caricis* on hypertrophied petiole or vein tissue discharged spores farther than those from unhypertrophied lamina. It is also significant that spores would not even fall out of aecia of *P. podophylli* on leaves on which numerous telia speeded evaporation, although they were ejected vigorously from the more turgid leaves that bore only the scattered aecia. Large spores also tend to be thrown further than small ones, which partly accounts

TABLE I

HORIZONTAL DISCHARGE DISTANCE OF AECIOSPORES WITH AND WITHOUT PORE PLUGS

Species	Maximum discharge distance (mm) in various tests
With plugs	
Puccinia caricis var. *grossulariata*	
lamina	4.5– 7.5
vascular tissue	10.5–12.0
P. extensicola var. *asteris*	7.0– 8.0
P. extensicola var. *oenotherae*	8.0
P. graminis	3.5– 7.0
P. helianthi	7.5– 9.0
P. podophylli, hypertrophied	11.0–13.5
P. rubigo-vera var. *impatientis*	6.0– 9.0
Without plugs	
P. andropogonis var. *pustulata*	3.0– 6.0
P. coronata	6.5– 9.5
P. mariae-wilsoni	3.0– 7.0
P. polygoni-amphibii var. *persicariae*	6.0– 8.5
P. violae	5.0– 6.5
Uromyces caladii	2.0– 6.0
U. fabae	2.0– 4.5

for the small discharge distance in *P. andropogonis* and for the great discharge distance in *P. podophylli*. The pore plugs are immersed to about half their diameter in the pores and the residual protuberance seems to be too slight to cause any useful invagination of the spore. It is doubtful whether the plugs have any significance other than being a by-product of pore formation; but their presence or absence seems to be constant in many species and should always be noted when aecia are examined.

Dodge's model seems to provide the key to aeciospore discharge, but the necessary invagination of the spore appears to be caused by the intercalary cell. When the aeciospore initial undergoes its final division

into spore and intercalary cell it is nearly cylindrical in form—a cylinder with its curved sides slightly flatened by contact with similar adjacent cylinders. If it were to divide into two equal parts it would probably do so by means of a plane, transverse septum, although the exact form and position of the septum would depend upon the proportions of the cell. But if, by we know not what controlling mechanism, the initial is to be cut into two very unequal parts the smaller part will be cut out of its corner; and, as Thompson (**25**, ch. 8) shows us from Berthold's examples, the septum of minimal area will be a curved one, theoretically meeting the existing walls at right angles. The reader will frequently see such curved septa at the bottom of a glass if he partakes of dish-washing. This condition is closely approached by the intercalary cell (FIG. 22), which is always offset and is cut off by a curved septum. Fixed preparations do not show the septum meeting the end wall of the initial at right angles, but this divergence from theory may be explained partly by distortion in processing and partly by forces at work within the cell. The point is that this curved corner partition gives the required invagination of the basal side of the spore with the minimum wastage of protoplasm. The offset position slightly reduces mechanical efficiency, but it is mathematically impossible for the required form of cell to be cut off symmetrically. Evidently the offset position is no mere accident. The apparent function of the intercalary cell as a disjunctor explains its persistence in the aeciospore chains despite its absence from the chains of peridial cells.

The intercalary cell is usually regarded as very ephemeral; but in living, turgid aecia it remains turgid until maturation of its companion spore. It is extremely thin-walled, no unnecessary substance being wasted upon it, and quickly collapses beyond recognition in dried material. It is obvious that the slightest reduction in turgor is likely to affect the outermost intercalary cells more than any other element in the spore chain. The complete cessation of spore discharge under such conditions becomes much easier to understand if the intercalary cell is assumed to play an active part.

The aeciospore plugs serve to indicate the positions and numbers of the germ-pores, usually invisible in unstained material unless the wall is pigmented. If chains of slightly immature spores are examined we may find that although the pattern varies from chain to chain, the pore positions are often very constant in a single chain.

Before leaving the aecium it is perhaps worth recollecting that the aecidioid peridial cell has its inner face much thinner than the outer one. Turgor consequently causes each cell to curve, like a stomatal guard

cell, and curl back the peridium out of the way of the discharging spores. In the rostelioid peridium the outer walls of the cells are the thinner; and turgor bows out the sides of the peridium, causing longitudinal slits to develop, through which the spores sift out.

PARTITIONING OF SPACE BY THE SPORES OF TOLYPOSPORIUM

The mathematical problem of the complete filling of space by similar, originally spherical cells is less frequently encountered in mycology than in the study of vascular plants. So-called pseudoparenchymatous tissue is generally composed of elongate mycelial cells variously compacted together. We often see spores compacted in crusts such as the telia of *Chrysomyxa* or *Melampsora*, in sheets such as the teliospores of some species of *Entyloma* form between the palisade cells of a leaf, or in spore balls such as those of *Thecaphora* or *Urocystis*. But in none of these examples do we find spores deep in a homogeneous tissue where they are exposed to symmetrical forces. As recently indicated (**20**), *Tolyposporium bullatum*, a smut attacking *Echinochloa*, has very large aggregations of relatively small spores, and is in a different category. When the spore balls are cleared it can be seen that little or no space is left between the spores. The spores are so firmly united that some of them break when a ball is crushed, leaving wall fragments adherent to their neighbors, which gave rise to the claim that the spores were decorated with high wings that interlocked to hold them together. If the balls are softened and broken up as gently as possible, to minimize spore breakage, we see that many spores are nearly spherical but covered by flat, polygonal facets. There are two well-known space-filling solids that might be expected to result from the compression of packed spheres. The rhombic dodekahedron, with twelve identical faces, was long held to be the most economical such solid, i.e. that which filled space with the minimum partitional area. It was later proved that the perfect space-filling solid is actually the tetrakaidekahedron, which has six square faces in opposite pairs and eight hexagonal faces, each square being bounded by four hexagons (**25**, ch. 7). When peas or clay pellets are packed symmetrically and compressed, dodekahedra result. Only when friction is reduced and the spheres slide freely upon each other do the figures approach the tetrakaidekahedron. A perfect tetrakaidekahedron is probably never formed in a tissue, but it is approached by some of the spores of *Tolyposporum bullatum*. The small size of spores makes exact analysis difficult, but one occasionally sees a square facet surrounded by four nearly perfect hexagons.

HELICAL STRUCTURE IN THE TELIOSPORE PEDICELS OF PHRAGMIDIUM

It was noted in cytological preparations of *Phragmidium americanum* that a helical structure was visible in the stained teliospore pedicels (FIG. 23). There was no immediate opportunity to look into the matter, which was later forgotten until after the war when Mr. A. Payette, then working in this laboratory, noticed in preparing a mount from a *Phragmidium* telium that the spores rotated about the long axes when the pedicels were moistened or were drying. This rotation is clearly due to the helical structure of the pedicel. Whether the rotation performs any useful function is not clear. It might serve to lift a basidium clear of a film of water; but, in our imperfect understanding of the forces involved, it seems just as likely that it might thrust the basidium under water. It is possible that growth or surface forces prevent such a submergence. It is also possible that the structure is functionless and is simply a result of the molecular structure of the pedicel wall; but such a structure has not been seen in other genera of rusts investigated. The helical striae appear to be of geodetic form, allowing the most efficient rotation of the pedicel.

CONSTRICTION AT TELIOSPORE SEPTA

If the cells of a teliospore behaved as fluid bubbles not subjected to any force but surface tension, the septa and the tangents to the outer walls would meet at equal angles of 120° (FIG. 24). We occasionally see approximately this degree of constriction in e.g. *Tranzschelia fusca* or some species of *Puccinia*. Constriction to a more acute angle is possible if growth is resumed after the septum has been formed and thickened, but is not frequently seen. It is to be expected only if the septum is formed before any thickening of the outer wall occurs; then, if the secondary thickening is laid down uniformly the septum will be almost twice as thick as the outer wall (ratio, primary + twice secondary : primary + secondary), as shown in FIG. 25. In such a spore the septum is much less elastic than the outer walls and will stretch little under pressure that bulges the cells. It should also be noted that in such a spore the two cells are held together only by the primary wall and often separate quite easily. On the other hand, if half of the secondary wall is laid down prior to septation and the remainder is uniformly deposited after the septum has been formed, the outer wall will be as thick as the septum (FIG. 26). Consequently constriction tends to be less and the continuous band of secondary wall reduces the likelihood of the cells breaking apart.

Thus we see that the tendency to separate is not governed by the deep constriction, but rather that both characters are due to delay in the deposition of the secondary wall. Both these characters show some degree of constancy in many species of rusts, but they are not always completely reliable as taxonomic aids; e.g. *Puccinia malvacearum* shows considerable variation even within a sorus. A more interesting example is *P. cutremae*, in which, as Jørstad (**13**) showed, the cells often separate freely. This tendency was used by Arthur (**1**) as a key character to separate *P. cutremae* from *P. oudemansii;* but in most specimens of *P. cutremae* from northern Canada no appreciable separation occurs. These closely related species can be distinguished by the wall sculpturing, but the tendency to separation is evidently not a reliable character.

CONVERGENT EVOLUTION AND THE SUPPOSED HOMOLOGY BETWEEN ASCUS HOOK AND CLAMP CONNECTION

Probably nothing has so seriously delayed our understanding of the phylogeny of the Basidiomycetes as the dogmatic assertion that the clamp connection of many Basidiomycetes is homologous with the somewhat similar ascus hook of the higher Ascomycetes. To cast doubt upon this cherished belief must appear heretical to many who, as undergraduates, were taught this assumption, bolstered by convincing although somewhat unrealistic diagrams.

To view the problem dispassionately we must discuss the important subject of convergent evolution, which has been too little considered by many mycologists. Convergent evolution is of great importance in many groups of plants and animals and there is no reason to suppose that the fungi are exempt. A few clear examples are given earlier in this paper, notably the repeated development of the deciduous teliospore pedicel and of various types of spore decoration.

The crux of the matter is that, if any problem is important to the survival of a group of organisms and there are only a few conceivable ways of solving it, the same solution will frequently be adopted more than once. It may be helpful if, before returning to the fungi, we consider a few incontrovertible and striking examples of convergence in other organisms.

In most birds, flight and feeding occupy many hours of every day. Because these processes are so important it is inevitable that many examples of convergence should be seen in wings and bills. The hooked bill, suitable for tearing flesh, has been developed independently in the diurnal raptores (hawks, eagles, falcons, etc.) and in the owls, which

are not closely related. The widely gaping bill, often subtended by bristles, has been developed repeatedly in unrelated groups that catch insects in flight, e.g. swifts, tyrant flycatchers, swallows, and old-world flycatchers. The heavy, conical, so-called finch bill, adapted to cracking seeds, has apparently been evolved at least three times, as Beecher (**2, 3**) has recently shown in careful studies employing highly conservative muscle characters. Several major types of wing form can be distinguished among birds, and some have been developed several times. The long soaring wing, impractical in land birds, has been developed repeatedly in oceanic birds. The high-speed wing has been fully developed in falcons, plovers and sandpipers, swifts and hummingbirds, and swallows; yet the skeletal structure is quite different in these groups, and the swifts and hummingbirds actually have a different flight mechanism from the others (**17**). Emarginated primary feathers provide a series of aerodynamic slots that increase lift when the wing is fully spread. They have been highly developed several times in large birds and in very active small birds that take off thousands of times a day. The most efficient form of slot, square instead of tapering at the base, is found in the two main, and unrelated, groups of predators, which must lift heavy loads. A vacant ecological niche may be filled repeatedly; thus blossom-visiting hummingbirds of the new world have their counterparts in the tiny sunbirds of Africa.

Among plants the dispersal of seeds or spores is vitally important. Almost every conceivable method of aiding dispersal has been developed, and many have occurred repeatedly. Plumed seeds or fruits, dispersed by wind, have developed independently in e.g. Cyperaceae (*Eriophorum*), Salicaceae, Rosaceae (*Geum, Dryas*), Onagraceae, Asclepiadaceae, and Compositae. The scape elongates markedly after anthesis, assisting escape of the plumed seed or fruit, in e.g. *Dryas drummondii* and *Taraxacum officinale*. Explosively dehiscent capsules occur in e.g. Leguminosae and Balsaminaceae. Slender plants, bearing erect capsules that open apically, and dispersing their seeds when they swing in the wind, occur in many families. In the shalter of woods or cliffs, where the wind is seldom effective, the kinetic energy of falling water may power the dispersal process. Thus the splash cup is found in fungi, bryophytes, and seed plants, in sheltered places where water falls vertically in large drops (**4, 5, 19**). This same force is used to power a more complex mechanism in *Tiarella* (**21**), and investigation may eventually show that other ways of using it have been developed. Hooked and retrorsely barbed seeds and fruits are common in various families of flowering plants, all similar in function but very diverse in

origin. The deflexed, slender perigynia of *Carex microglochin* and related species are an efficient device for transportation by birds and mammals (**22**); in *Triglochin maritima* the valves of the capsules split away from the base, providing exactly the same device by a different means. The samara of *Acer*, with its rigid wing thickened at one edge, is an efficient device for reducing rate of fall; it is exactly duplicated functionally, but not anatomically, in *Pinus* and in *Mansonia* (Sterculiaceae) (**26**), and was finally "evolved" by man in world war II as a substitute for the parachute in dropping small pieces of freight.

In the hope that these examples, turned up without any real search from a few groups of organisms with which I have some familiarity, will satisfy the reader that convergent evolution is far from rare, we may now return to the fungi. I have little firsthand knowledge of the Hymenomycetes, but from what I read and from discussions with students of this group I find it difficult to believe that current classifications based essentially on hymenophore conformation can be even approximately natural. The growing mass of biochemical evidence throws more and more doubt upon the reality of some groupings. The situation has recently been summarized by Imazeki (**12**) who states: "It is logical to say that the configuration of the hymenophore does not represent a phylogenetic difference between the higher taxonomic groups, but merely a lower difference within each taxonomic group." It is obviously advantageous to the fungus if the area of the hymenium can be increased without increased size of the fruit body. Such an increase can be achieved by folding or tuberculation, from which may develop gills, tubes, or teeth. With relatively few available choices it need not surprise us if some of these devices have been utilized more than once.

Hughes (**10**) points out that superficially similar slimy heads of hyaline amerospores, on mononematous, dematiacous conidiophores, must actually be divided among four major sections of the Hyphomycetes, on the basis of fundamental morphology. These slimy spores are evidently adapted to transportation by insects that are attracted to the decaying bark or wood on which these fungi grow (Hughes, personal communication). Detailed consideration of any group of fungi would inevitably yield more instances of convergence, but these examples will suffice.

We may now reconsider the supposed homology of ascus hook and clamp connection. In any dikaryon it is essential that, after each simultaneous division of the nuclei, regrouping to bring compatible pairs together must be achieved without undue waste. This end may be achieved by the cells being broad enough for the division spindles to be

the side by side, which occurs in e.g. the sporogenous columns of the rust aecium and in the contexts of some mushrooms; but this is too wasteful a process for distributive mycelium, since it requires that the cross-sectional area of each cell must be nearly four times that needed to accommodate individual spindles. Alternatively, after each division of the dikaryon the two central nuclei of the four may slide past each other, as happens regularly in the distributive hyphae of the rusts (**16**) and presumably in *Taphrina*. I find it difficult to imagine any other satisfactory means of maintaining the dikaryon without some such device as the ascus hook or clamp connection. With only three conceivable methods of bringing together the compatible nuclei, it can hardly be considered remarkable if all three methods have been used in more than one group of organisms.

If we admit that the crozier and clamp connection are not necessarily homologous, it at least becomes possible to draw up a rational evolutionary scheme for the Basidiomycetes; for we are then able to search for their origins among the older, generalized Ascomycetes that can logically be expected to give rise to new major groups, instead of among climax groups, which are never sufficiently plastic for such a rôle. The beginnings of such an evolutionary scheme will be traced in a later paper.

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SUMMARY

This discussion does not cover the whole field of cellular mechanics, but deals with the following topics: basidiospore formation, teliospore evolution in *Puccinia*, effect of pressure upon teliospore shape, spore wall ornamentation, aeciospore discharge, spore form in *Tolyposporium*, pedicel structure in *Phragmidium* teliospores, constriction at teliospore septa, and the supposed homology between the ascus hook and clamp connection. It is shown that convergent evolution has been overwhelmingly important in evolution of the teliospores of *Puccinia* and *Uromyces* and in the development of the various types of spore wall ornamentation. Its occurrence elsewhere in the fungi is indicated and examples are given from other groups of organisms to illustrate the circumstances

under which it may be expected to operate. The rearrangement of nuclei after division of a dikaryon is of such importance and is capable of so few satisfactory solutions that the situation is extremely favorable to convergent evolution; and it is considered that acceptance of the ascus hook and clamp connection as homologous is not warranted.

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SOME HYPHOMYCETES THAT CAPTURE EELWORMS IN SOUTHERN STATES

CHARLES DRECHSLER [1]

(WITH 6 FIGURES)

Three clampless nematode-capturing hyphomycetes that came to light in Petri plate cultures planted with small quantities of decaying vegetable material obtained from Florida and Louisiana during the winter of 1952–1953 are herein described as new members of the genera *Arthrobotrys* Corda and *Dactylella* Grove. As these fungi were not observed in the numerous cultures I prepared during the last 20 years with slowly decomposing detritus from the District of Columbia and from various localities in the adjacent regions of Maryland and Virginia, there is reason to presume that their distribution in the United States extends mainly in our more southern latitudes. In capturing eelworms one of the three species employs solitary adhesive cells which, unlike the stalked adhesive cells of several related species described earlier, are attached directly to the mycelial hyphae. Adhesive cells of similar positional relationship are made known also in an amoeba-capturing fungus that from its commonplace cross-walls would seem referable to the same predacious series.

Arthrobotrys anchonia sp. nov.

Mycelium saepe sparsum; hyphis hyalinis, ramosis, mediocriter septatis, plerumque 2–5 μ crassis, hic illic ex ramulis bilocularibus 7—15 μ (raro usque 25 μ) longis et 4–6 μ crassis laqueos circulares 20–42 μ latos ferentibus qui in 3 cellulis arcuatis consistunt et aperturam rotundam vel rotundo-triangulam 12–32 μ latam circumdant; cellulis arcuatis 15–35 μ longis, extremo 2.8–4.7 μ crassis, medio 4–7.5 μ crassis; vermiculo nematoideo in laqueum apertum errato omnibus tribus cellulis abrupte se contrahentibus, animal magnopere comprimentibus, id ita necantibus, integumentum ejus perforantibus, hyphas intus evolventibus quae carnem exhauriunt; hyphis assumentibus mox mediocriter septatis, ex magna parte 2.5–4.2 μ crassis sed interdum in tubera 5–8 μ crassa abeuntibus. Hyphae fertiles incoloratae, erectae, saepius 3–8-septatae, 350–500 μ altae, basi 4–6 μ crassae, sursum leniter attenuatae, prope apicem 2.5–3.5 μ crassae, apice usque 8 (saepe 3–5) conidia in uno capitulo

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ferentes; conidiis incoloratis, elongato-obovoideis, plerumque 29–43 μ (saepius circa 35 μ) longis, 15–19 μ (saepius circa 16.8 μ) crassis, raro biseptatis, vulgo uniseptatis denique cellula inferiore plerumque 8–18 μ (saepe circa 11.5 μ) longa et cellula superiore plerumque 17–27 μ (saepe circa 23.5 μ) longa.

Vermiculos nematoideos diversos capiens consumensque habitat in materiis plantarum putrescentibus prope Laplace, Louisiana, etiam in caulibus radicibusque *Phaseoli vulgaris* putrescentibus prope Fort Lauderdale, Florida.

Mycelium on nematode-infested substrata usually scanty, spreading; vegetative hyphae colorless, septate at moderate intervals, mostly 2 to 5 μ wide, often (especially in presence of nematodes) giving rise on straight or curving stalks to circular rings in perpendicular positions; the stalks 7 to 25 μ (commonly 7 to 15 μ) long, 4 to 6 μ wide, usually composed of 2 unequal cells, the proximal cell 2 to 8 μ (mostly 2.5 to 5 μ) long, the distal cell 5 to 17 μ (mostly 5 to 12 μ) long; the rings, measuring 20 to 42 μ in diameter and surrounding a circular or rounded triangular aperture 12 to 32 μ wide, being regularly composed of 3 arcuate segments 15 to 35 μ long, 2.8 to 4.7 μ wide at the ends and 4 to 7.5 μ wide in the middle—the first and third segments being united to one another as well as to the end of the stalk; on entrance of a nematode into the aperture the arcuate cells contracting abruptly, all three indenting the animal broadly and deeply, thereby strongly constricting it and soon disabling it, then perforating its integument and extending through its body assimilative hyphae to appropriate its fleshy contents; the assimilative hyphae becoming septate at moderate intervals, for the most part 2.5 to 4.2 μ wide but often terminating in one or more bulbous enlargements or swollen cells 5 to 8 μ wide. Conidiophores colorless, erect, in later stages containing 3 to 8 cross-walls, 350 to 500 μ high, 4 to 6 μ wide at the base, tapering gradually upward to a width of 2.5 to 3.5 μ, at the tip often furnished with several short stout spurs on which are borne collectively 3 to 8 conidia in a handsome head; conidia colorless, elongate obovoid, mostly 29 to 43 μ (average 35 μ) long, 15 to 19 μ (average 16.8 μ) wide, rarely biseptate, usually uniseptate with the proximal cell commonly 8 to 18 μ (average 11.5 μ) long and the distal cell commonly 17 to 27 μ (average 23.5 μ) long.

Capturing and consuming nematodes of different species, it occurs in decaying plant residues near Laplace, Louisiana, and also in decaying stems and roots of *Phaseolus vulgaris* L. near Fort Lauderdale, Florida.

The bean (*Phaseolus vulgaris*) material from which *Arthrobotrys anchonia* was obtained came from young plants affected with basal stem rot and root rot, which were collected in southern Florida on January 13, 1953. When portions of softened cortex from the diseased seedlings were placed on Petri plates of maizemeal agar, mycelium of several species of *Pythium*, including especially *P. spinosum* Sawada, grew out promptly over the substratum. Soon the cultures became abundantly

infested with eelworms. About 10 days after the cultures had been started these animals began to suffer wholesale destruction by predacious hyphomycetes. In the beginning the eelworms were captured for the most part in the adhesive networks of *A. oligospora* Fres. and *A. musiformis* Drechsl. (**3**: 477–482). Somewhat later—in many instances about 15 days after the cultures were prepared—hyphae furnished with constricting rings were found spreading through the agar substratum, with the result that an increasing proportion of nematodes subsequently succumbed in the strangulating organs. Some tracts of annulated mycelium ultimately gave rise to conidiophores with solitary heads of slender uniseptate conidia typical of my *A. dactyloides* (**3**: 482–487), while other tracts produced conidiophores with solitary heads of conidia which, though also uniseptate, were markedly widened at the distal end, so that with respect to shape they seemed generally intermediate between the obovoid conidia of *A. oligospora* and the tapering obconical conidia of my *A. conoides* (**3**: 473–477).

The more robust form developed also in more than a dozen maizemeal agar plate cultures that after being overgrown by *Pythium ultimum* Trow had been further planted with small quantities of decaying vegetable detritus collected on December 20, 1952, near Laplace, Louisiana. In these cultures it again made its appearance rather tardily, after the number of infesting nematodes had been greatly reduced through capture by *Dactylaria haptospora* Drechsl. (**4**: 456–461), a knob-bearing species apparently very abundant in southern Louisiana, and by the familiar retiary species *A. oligospora*, *A. conoides*, and *A. musiformis*. As in the cultures prepared with Florida material it sometimes formed conidial apparatus at no great distance from *A. dactyloides*, though among strangulating species it was accompanied most often by my *Dactylella aphrobrocha* (**8**: 12–22). When its obovoid conidia were removed from the lofty conidiophores by touching them with a slab of sterile agar held on a flamed platinum spatula, and were then transferred to tubes of sterile maizemeal agar, pure cultures were readily obtained.

Growing undisturbed on maizemeal agar in the absence of alien organisms *Arthrobotrys anchonia* ordinarily produces a septate mycelium wholly devoid of predacious organs. If a block of agar permeated with such commonplace mycelium is removed to a Petri plate culture well infested with eelworms the new hyphae soon extended radially from the transferred mass will form constricting rings at intervals some distance backward from the advancing tip. The rings and their supporting stalks, as in other constricting species, are developed in planes at ap-

proximately right angles to the parent hyphae (FIG. 1, *A–C;* FIG. 2, *A, B*). Accordingly, in their original positions, they most often are directed edgeways to the observer, so that their cellular make-up is not well displayed. Their structure is shown more advantageously when they have been pushed sideways (FIG. 2, *C*) by passing eelworms, and is most clearly revealed in the occasional instances where under the impact of robust nematodes they have been bustled into nearly horizontal positions (FIG. 1, *D–K;* FIG. 2, *D, E*). Viewed flatways the rings strongly resemble those of *A. dactyloides* and *Dactylaria brochopaga* Drechsl. (**3**: 514–518). As in these species the two-celled stalk is of the relatively short, stout type wherein the basal cell is generally shorter than the distal cell, rather than of the longer slender type usual in *Dactylella aphrobrocha* and *Dactylella heterospora* Drechsl. (**5**), where the basal and distal cells are of approximately equal length. Because of considerable variability in the relative position of the septum dividing the stalk in *A. anchonia,* the basal cell here is sometimes scarcely one-third (FIG. 1, *D;* FIG. 2, *A, E*) and sometimes is more than one-half (FIG. 1, *I*) the length of the distal cell. Its length sometimes is greater (FIG. 1, *I;* FIG. 2, *D*) and sometimes is noticeably less (FIG. 1, *F, H;* FIG. 2, *A, B, C, E*) than its width. It is, however, not usually shortened as pronouncedly as the proximal stalk-cell of my *Dactylella bembicodes* (**3**: 487–492), which sometimes is found reduced to a comparatively thin plano-convex disc.

In *Arthrobotrys anchonia,* much as in *A. dactyloides,* the three arcuate cells that make up the constricting ring have no median protrusion on the side bordering the aperture, so that the aperture lacks the trefoil modification present, for example, in the predacious organs of *Dactylella heterospora.* Here and there rings may be found to have closed emptily (FIG. 1, *L*), but as a rule closure takes place in effecting capture of an eelworm (FIG. 2, *F, a, b*). After its integument has been narrowly perforated the hapless captive is invaded forward and backward by one or more assimilative hyphae (FIG. 1, *M, N;* FIG. 2, *G, H*). These hyphae, as in *A. dactyloides,* often become enlarged at the tip. In some instances not only the terminal segment, but also the penultimate segment undergoes marked enlargement (FIG. 1, *M*). Gradually all fleshy parts of the nematode disappear. The assimilative hyphae show increasing vacuolation and are progressively emptied as the protoplasmic contents migrate backward through the cells of the contracted ring and its stalk into the parent hypha. Ultimately these cells are in turn evacuated, and their membranous envelopes, like those of the assimilative hyphae and like the animal's integument, vanish from sight, leaving

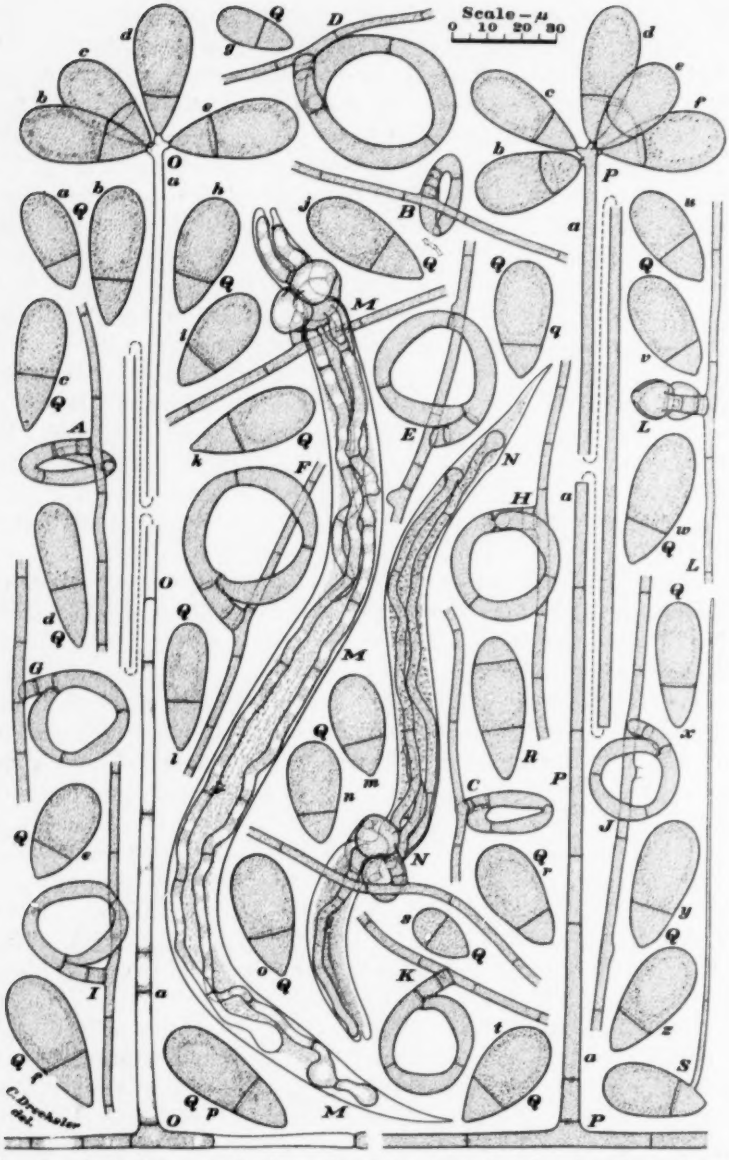


FIG. 1. *Arthrobotrys anchonia* as found in nematode-infested maizemeal-agar culture; × 500. *A–C*, Portions of hyphae, each bearing an open constricting ring in normal posture. *D–K*, Portions of hyphae, each with an open constricting ring

only a slightly protuberant irregularity in the contour of the mycelial filament to mark the earlier attachment of a constricting ring that had operated successfully in the destruction of prey.

Thus nourished through the capture of eelworms the fungus gives rise from procumbent mycelial filaments to erect conidiophores which sometimes attain a height of 0.5 mm, but more often are found measuring 0.4 to 0.45 mm in length. The individual conidiophore (FIG. 2, *I*, *a*) first produces a single conidium (FIG. 2, *I*, *b*) on its tip. In some instances no further development ensues, but much more often additional conidia are produced on short stubby spurs (FIG. 2, *J*), so that many conidiophores (FIG. 1, *O*, *a*; *P*, *a*) eventually bear aloft 4 (FIG. 1, *O*, *b–e*) or 5 (FIG. 1, *P*, *b–f*) conidia in a handsome cluster. Heads of 6, 7, or 8 conidia are formed less often, or, at least, were less frequent in my cultures. As in *A. dactyloides* and *A. musiformis* the conidiophores of the fungus are not given to repeated elongation, but regularly terminate their development with the formation of a single spore cluster.

Because of similarities in its monocephalous sporulating habit as well as in the morphology of its predacious rings *Arthrobotrys anchonia* must be held related most closely to *A. dactyloides*, though, as has been noted, its distally widened, somewhat obovoid conidia (FIG. 1, *Q*, *a–z*. FIG. 2, *K*, *a–z*; *L*, *a–e*) are reminiscent rather of *A. oligospora* and *A. conoides*. Parallelism with the latter two species and divergence from *A. dactyloides* are evident not only in the outward shape of its conidia but also in their unequal partitioning into a small basal cell and a much larger distal cell. According to the metric data given in the diagnosis the distal cell in *A. anchonia* would seem about twice as long as the basal cell; and further, on the average, would seem twice the width of the distal cell in the medially uniseptate conidia of *A. dactyloides*. The metric data in question were derived from measurements of 100 conidia selected at random in mounts prepared from nematode-infested agar plate cultures. The 100 measurements for length, expressed in the nearest integral number of microns, were distributable as follows: 29 μ, 2; 30 μ, 2; 31 μ, 4; 32 μ, 11; 33 μ, 12; 34 μ, 14; 35 μ, 16; 36 μ, 8; 37 μ,

turned flatwise to the observer. *L*, Portion of hypha with a constricting ring that has closed emptily. *M*, *N*, Portions of hyphae, each with a constricting ring that after closing on an eelworm (*Eucephalobus* sp.) has extended assimilative hyphae through the captive. *O*, Prostrate hypha with an erect conidiophore, *a*, that bears 4 conidia, *b–e*. *P*, Prostrate hypha with an erect conidiophore, *a*, which supports 5 conidia, *b–f*, in a head. *Q*, Detached uniseptate conidia, *a–z*, showing variations in shape, size, and position of cross-wall. *R*, Biseptate conidium. *S*, Conidium with slender filamentous aerial outgrowth. (Owing to lack of space *O* and *P* are shown in parts whose proper connection is indicated by broken lines.)

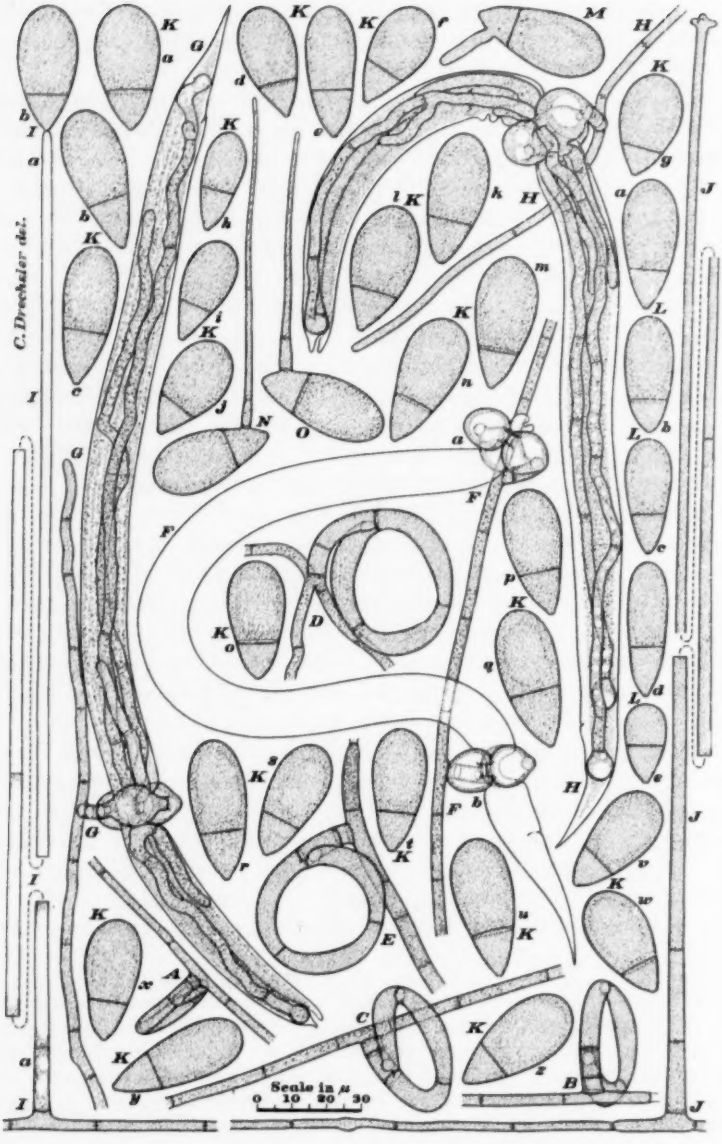


FIG. 2. *Arthrobotrys anchonia* as found in nematode-infested maizemeal-agar culture; × 500. *A, B,* Portions of hyphae, each with an open constricting ring in normal posture. *C,* Portion of hypha with an open ring turned slightly sideways.

11; 38 μ, 13; 39 μ, 3; 40 μ, 2; 41 μ, 1; 43 μ, 1; while the measurements for greatest width were distributable thus: 15 μ, 5; 16 μ, 28; 17 μ, 46; 18 μ, 20; 19 μ, 1. When many hundreds of conidia were examined some exceptionally large individuals as much as 45 μ long, or as much as 20 μ wide, came under observation, together with some undersized individuals (FIG. 1, *Q*, *g*, *s*. FIG. 2, *K*, *h*; *L*, *e*) not greatly beyond the dimensional range of my *A. cladodes* (**3**: 459–464). A few biseptate specimens were also found, their 2 cross-walls usually delimiting a large median cell from a smaller basal and a smaller apical cell (FIG. 1, *R*).

These few biseptate spores, no less than the obovoid uniseptate conidia normally formed by *Arthrobotrys anchonia*, resemble somewhat the distended conidia produced now and then by *A. dactyloides* (**3**: 484–485, 537, f. 18, *K*, *a–p*). Production of distended conidia, however, can not well be considered typical of *A. dactyloides*, as it takes place only sparingly in nematode-infested cultures, though often occurring somewhat abundantly when robust mycelium of the fungus that has developed in pure culture on rich maizemeal agar becomes heavily infested with mites. Yet production of distended conidia by *A. dactyloides*, even in meager quantity, would make difficult any recognition of *A. anchonia* as a separate species if the latter fungus were distributed, like the former, in our middle and northern latitudes as well as in our southern states.

Conidia of *Arthrobotrys anchonia* germinate readily on moist fresh substratum by putting forth one or more vegetative germ hyphae (FIG. 2, *M*). In aging cultures they often extend a slender outgrowth vertically in the air (FIG. 1, *S*; FIG. 2, *N*, *O*).

Dactylella megalospora sp. nov.

Mycelium effusum; hyphis sterilibus incoloratis, ramosis, mediocriter septatis, plerumque 2–6 μ crassis, saepe laqueos tenaces arcuatos vel circulares in reticula interdum conjunctos proferentibus; his laqueis reticulisque vermiculos nematoideos illaqueantibus, deinde tum integumentum animalis captivi anguste perforantibus,

D, *E*, Portions of hypha, each with an open ring turned flatwise. *F*, Portion of hypha with 2 rings, *a* and *b*, that have closed in capturing a single eelworm (*Eucephalobus* sp.). *G*, *H*, Portions of hyphae, each with a closed ring that holds a captured eelworm (*Eucephalobus* sp.); from each ring assimilative hyphae have been extended lengthwise through the captive. *I*, Prostrate mycelial hypha with an erect conidiophore, *a*, bearing a single conidium, *b*. *J*, Procumbent hypha with erect conidiophore denuded of its spores. *K* (*a–z*), *L* (*a–e*), Detached uniseptate conidia showing variations in size, shape, and position of cross-wall. *M*, Detached conidium from which a vegetative germ tube is being extended. *N*, *O*, Detached conidia, each with an erect slender filamentous aerial outgrowth. (Owing to lack of space *I* and *J* are shown in parts whose proper connection is indicated by broken lines.)

tuber mortiferum globosum intrudentibus, hyphas intus evolventibus quae carnem exhauriunt. Hyphis fertilibus incoloratis, erectis, simplicibus vel interdum breviramosis, plerumque 1–7-septatis, 150–450 μ altis, basi vulgo 6–8 μ crassis, sursum leniter attenuatis, prope apicem saepe 2–3 μ crassis, unum conidium vel interdum aliquot conidia ferentibus; conidiis incoloratis, late fusoideis vel elongato-ellipsoideis vel obovoideis, plerumque 40–75 μ longis, 18–35 μ latis, 2–5-septatis, una cellula eorum (in late fusoideis quadriseptatis solitariis conidiis saepissime cellula media) multo longiore et latiore quam aliis cellulis.

Vermiculos nematoideos capiens consumensque habitat in radicibus et caulibus *Phaseoli vulgaris* putrescentibus prope Fort Lauderdale, Florida.

Mycelium spreading; vegetative hyphae colorless, branched, septate at moderate intervals, mostly 2 to 6 μ wide, often (especially in the presence of nematodes) giving rise to arched or circular hyphal meshes, which, though at first discrete, are later frequently compounded into more or less extensive networks; the meshes and networks capturing nematodes through adhesion and entanglement, then narrowly perforating the integument of each captured animal and intruding one or more infective bodies of subspherical shape, from which are extended assimilative hyphae, mostly 2 to 5 μ wide, that appropriate the fleshy contents. Conidiophores colorless, erect, often containing 1 to 7 cross-walls, commonly 6 to 8 μ wide at the base, tapering gradually upward to a distal width of 2 to 3 μ, when developed on nematode-infested substratum usually 350 to 450 μ high, unbranched and bearing a single conidium, but when developed on an artificial nutrient substratum often only 150 to 250 μ high, and then frequently bearing mostly near the apex 1 to 5 spurs usually 10 to 40 μ long, on each of which, as on the axial tip, a conidium is produced. Conidia colorless, broadly fusoid or elongate-ellipsoidal or obovoid, mostly 40 to 75 μ long and 18 to 35 μ wide, commonly divided by cross-walls into 3 to 6 cells one of which greatly exceeds the others in length and width; the large cell somewhat variable in position, but occurring most often in median position in the especially distinctive large, broadly fusoid, quadriseptate conidia, frequently 55 to 75 μ long and 23 to 35 μ wide, that are produced singly on nematode-infested substratum.

Capturing and consuming motile nematodes, it occurs in decaying roots and stems of *Phaseolus vulgaris* near Fort Lauderdale, Florida.

The bean material to which reference is made in the foregoing diagnosis was taken from several diseased seedlings kindly collected by Dr. W. D. Moore in southern Florida on January 20, 1953. When the softened bean roots and bean stems were put on Petri plates of maize-meal agar, mycelium of my *Pythium myriotylum* promptly grew out in every instance. The resulting cultures permitted abundant multiplication of eelworms and thereby soon afforded opportunity for copious development of several predacious hyphomycetes, including *Arthrobotrys*

oligospora, *A. musiformis*, and *A. dactyloides*. After the eelworm population had suffered marked reduction, *Dactylella megalospora* somewhat belatedly came under observation. Its adhesive networks (FIG. 3, *A*), formed at wide intervals on long mycelial filaments, conformed closely to the more usual type of retiary apparatus exemplified in *A. oligospora*, showing no departures in design or dimensions such as distinguish the networks of *A. musiformis* and *Dactylaria polycephala* Drechsl. (**3**: 527–531). In *A. megalospora*, as in other retiary species, capture of eelworms was effected by entanglement and adhesion (FIG. 3, *B*). Narrow penetration of the captive's integument, intrusion of one or more infective bodies, and extension of assimilative hyphae lengthwise through the fleshy interior (FIG. 3, *C*) ensued in familiar sequence.

Evidently obtaining its nourishment wholly by the destruction of eelworms, *Dactylella megalospora* gave rise in the cultures started from Florida bean material to rather sparsely scattered conidiophores (FIG. 4, *B, a; C, a*) about 400 μ in height, each supporting aloft a single conidium (FIG. 4, *B, b; C, b*). The conidia thus produced were predominantly of broadly fusiform shape and most often consisted of 2 small proximal cells, a large dolioform median cell, and 2 small distal cells (FIG. 4, *D–S*). In their symmetry and cellular make-up they resembled especially the conidia of *Dactylella aphrobrocha* and *Dactylella coelobrocha* Drechsl. (**7**). With respect to outward form they appeared intermediate between the conidia of the two strangulating fungi, being prolonged at the narrowing basal and distal ends more strongly than the conidia of *D. aphrobrocha*, but less strongly than the conidia of *D. coelobrocha*. Twenty-five of the solitarily produced, quadriseptate conidia, selected at random, gave measurements for total length that ranged from 57.5 to 72 μ, and averaged 65.3 μ; while the measurements for greatest width ranged from 24 to 35 μ, and averaged 30.8 μ. The component cells of the 25 spores gave measurements for length with ranges and averages as follows: basal cell, 9.4 to 13.5 μ, 11.2 μ; parabasal cell, 5.2 to 7.3 μ, 6.3 μ; median cell, 29.2 to 39.4 μ, 34.7 μ; penultimate cell, 4.2 to 6.8 μ, 5.5 μ; apical cell, 4.2 to 9.4 μ, 7.6 μ. The basal cell here clearly exceeded the apical cell in length, whereas in the quadriseptate conidia of *D. coelobrocha* the apical cell appears generally longer than the basal cell. Very often the delimiting wall at the relatively narrow, somewhat truncate basal end was found modified by a lump-like deposit of opaque material—a modification usually absent in allied species.

Conidia diverging from the predominant symmetrically quadriseptate type were not scarce in the nematode-infested cultures where *Dactylella megalospora* was first discovered. Triseptate specimens occurred there

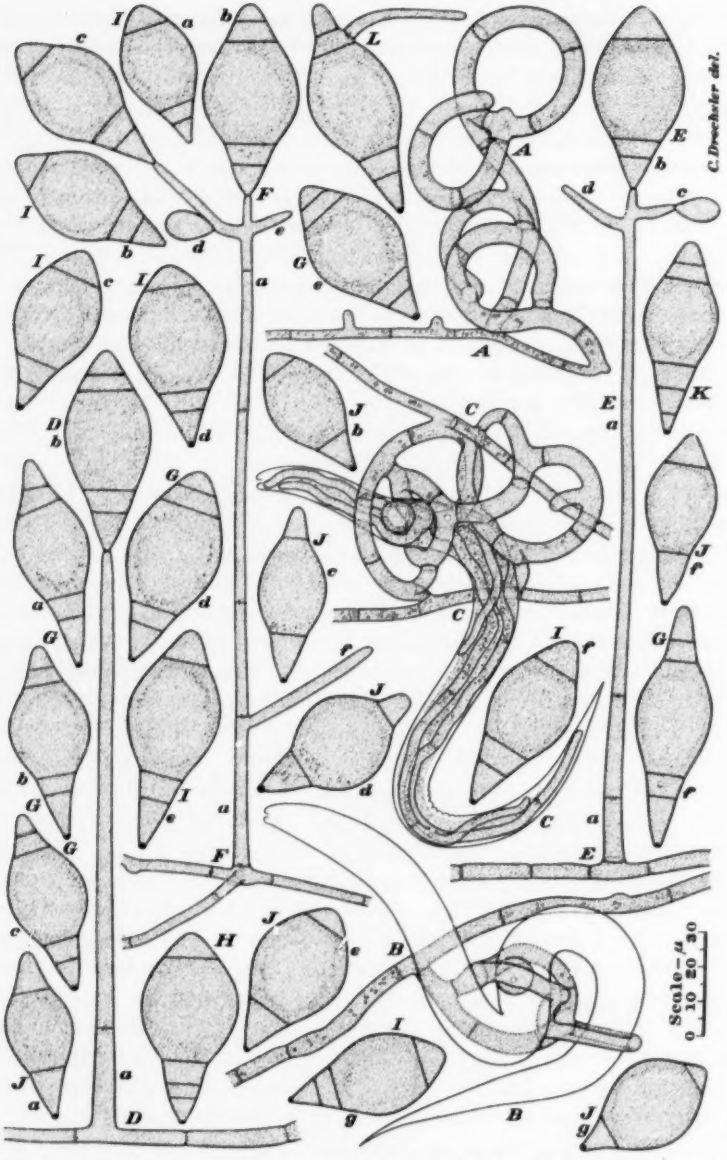


FIG. 3. *Dactylella megalospora* as found on nematode-infested substratum (*A–C*) and on slabs taken from maizemeal agar cultures (*D–L*); × 500 throughout. *A*, Hyphal network of several meshes. *B*, Small hyphal network with a captured

in considerable numbers, some showing the distended cell in penultimate position (FIG. 4, *T*), others showing it in parabasal position (FIG. 4, *U*). Biseptate specimens also occurred, and in these the distended cells were sometimes found in terminal position (FIG. 4, *V*).

Dactylella megalospora was isolated without difficulty by removing its conidia from the tall conidiophores to tubes of sterile maizemeal agar, the removal being accomplished by means of a sterile agar slab held on a flamed platinum spatula. In the absence of alien organisms the fungus generally elaborates no predacious networks. However, when blocks of agar well permeated with mycelium are excised from a pure culture and placed on nematode-infested substratum, adhesive meshes and networks will usually be formed not only on the new hyphae that soon grow out somewhat radially but also on many of the older hyphae (FIG. 4, *A*) that were present in the agar block at the time it was transferred.

The conidiophores produced by *Dactylella megalospora* in pure culture often attain only half the usual height of those developed in nematode-infested cultures. Some of these short conidiophores are unbranched (FIG. 3, *D*, *a*) and bear a single conidium (FIG. 3, *D*, *b*). Others (FIG. 3, *E*, *a*; *F*, *a*), in addition to bearing a conidium (FIG. 3, *E*, *b*; *F*, *b*) on the axial tip, produce one (FIG. 3, *E*, *c*) or more conidia (FIG. 3, *F*, *c*, *d*) on lateral spurs. Owing perhaps to the narrow attachment of the conidia many spurs (FIG. 3, *E*, *d*; *F*, *e*, *f*) are rather soon left denuded. In general the conidia formed in pure culture appear smaller and more variable with respect to size, shape, and septation than those formed on nematode-infested substratum. Their length commonly ranges from 40 to 60 μ and their width from 17 to 25 μ. The quadriseptate individuals among them, with the distended cell frequently in median (FIG. 3, *G*, *a–f*) or penultimate (FIG. 3, *H*) position, are sometimes not more numerous than the triseptate (FIG. 3, *I*, *a–g*) or biseptate (FIG. 3, *J*, *a–g*) individuals. Quinqueseptate individuals (FIG. 3, *K*) are produced only in small numbers. Detached conidia germi-

eelworm into which a globose infective body has been intruded. *C*, Hyphal network with a captured eelworm showing 3 globose infective bodies from which assimilative hyphae have been extended forward and backward. *D*, Portion of hypha with a short unbranched conidiophore, *a*, bearing a single conidium, *b*. *E*, Portion of hypha with short conidiophore, *a*, bearing 2 conidia, *b* and *c*, as well as a denuded subapical spur, *d*. *F*, Portion of mycelium with short conidiophore, *a*, supporting 3 conidia, *b–d*, and 2 denuded spurs, *e* and *f*. *G*, Detached quadriseptate conidia, *a–f*, in which the median cell is largest. *H*, Quadriseptate conidium in which the penultimate cell is largest. *I*, Triseptate conidia, *a–g*, in which the penultimate cell is largest. *J*, Biseptate conidia, *a–g*, in which the median cell is largest. *K*, Quinqueseptate conidium in which the antepenultimate cell is largest. *L*, Germinating quadriseptate conidium.

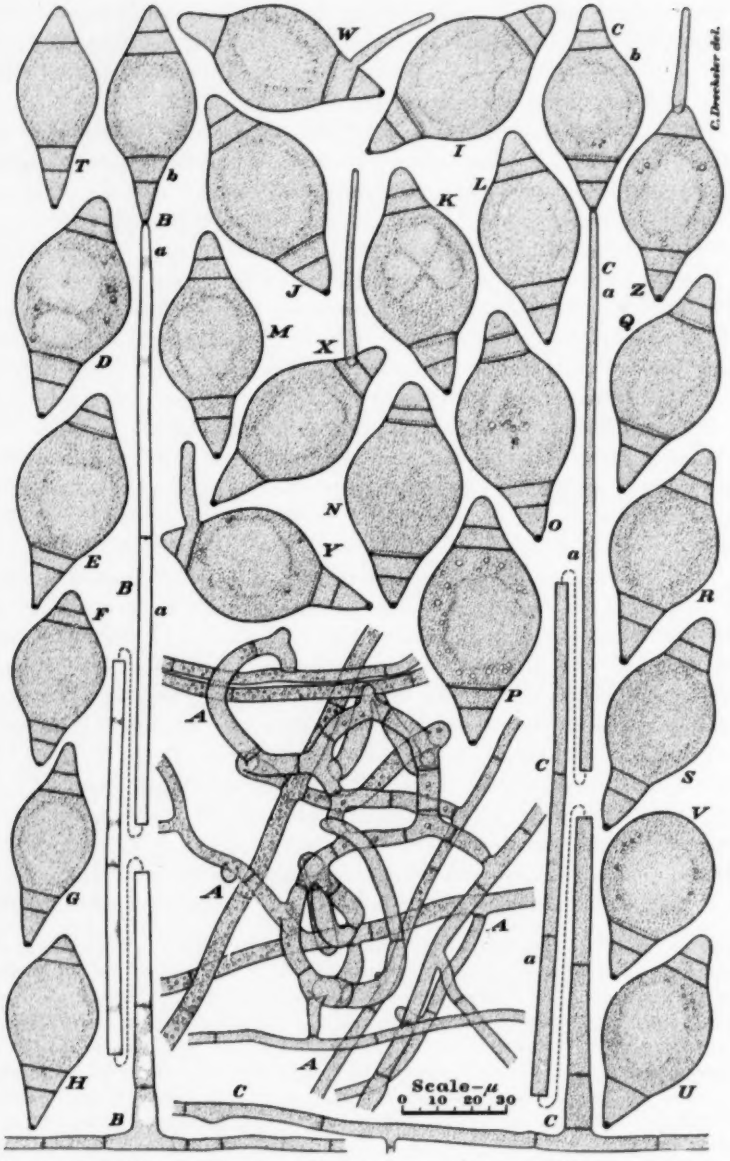


FIG. 4. *Dactylella megalospora* as found on agar slab removed from pure culture to nematode-infested substratum (*A*), and as appearing commonly when development has been wholly on nematode-infested substratum (*B–Z*); × 500 throughout.

nate rather freely both in pure culture (FIG. 3, *L*) and on nematode-infested substratum (FIG. 4, *W–Z*), the germ-tube either developing as a delicate aerial outgrowth (FIG. 4, *X*) or as a more robust vegetative hypha (FIG. 4, *Y*).

Dactylella megalospora apparently has its closest known relatives in the retiary species I described under the binomials *Dactylaria thaumasia* (**3**: 518–523), *Dactylaria psychrophila* (**6**: 154–166) and *Dactylaria cudermata* (**8**: 31–41). While its taxonomic affinities might have been indicated somewhat better by assigning it likewise to *Dactylaria*, such assignment seems at present hardly justified by the slight tendency toward capitate sporulation hitherto shown by the new fungus. Its conidia resemble those of *Dactylaria psychrophila* in their decidedly large dimensions as well as in their frequently quadriseptate partitioning, but differ rather markedly in their strongly ventricose shape resulting from pronounced distention usually of the median cell and less often of the parabasal or penultimate cell.

Dactylella phymatopaga sp. nov.

Mycelium sparsum; hyphis sterilibus incoloratis, ramosis, mediocriter septatis, plerumque 1.8–3.5 μ crassis, hic illic saepe tuberibus tenacibus praeditis; tuberibus tenacibus obovoideis vel elongato-ellipsoideis, plerumque 6–9.5 μ longis, basi 2–3 μ crassis, sursum 3.8–5.5 μ crassis, prope basim vulgo hyalinis sed in parte ulteriore saepissime protoplasmatis granulosi repletis, saepe ad vermiculos nematoideos inhaerentibus itaque animalia tenentibus, integumentum eorum perforantibus, bullam debilitantem intrudentibus, hyphas intus evolventibus quae carnem exhauriunt; hyphis assumentibus mox mediocriter septatis, plerumque 2–4.5 μ crassis; hyphis fertilibus incoloratis, erectis, 3–9-septatis, saepius 250–325 μ altis, basi 3.5–4.5 μ crassis, sursum leniter attenuatis, apice circa 2 μ crassis, ibi unum conidium ferentibus; conidiis hyalinis, vulgo fusoideis, basi truncatis, apice rotundatis, plerumque 40–60 μ longis, 11–18 μ crassis, vulgo quadriseptatis, cellula eorum media (antepaenultima) longiore et crassiore quam aliae cellulae.

Vermiculos nematoideos (Aphelenchoideum subtenuem inter alios) capiens consumensque habitat in materiis plantarum putrescentibus prope Laplace, Louisiana.

Mycelium often scanty; vegetative hyphae colorless, branched, septate at moderate intervals, mostly 1.8 to 3.5 μ wide, often (especially in the presence of nematodes) bearing adhesive protuberances at intervals of

B, *C*, Portions of hyphae, each with unbranched conidiophore, *a*, bearing a single conidium, *b*. *D–S*, Quadriseptate conidia in which the median cell is largest. *T*, Triseptate conidium in which the penultimate cell is largest. *U*, Triseptate conidium in which the antepenultimate cell is largest. *V*, Biseptate conidium in which the apical cell is largest. *W–Z*, Germinating conidia. (Owing to lack of space *B* and *C* are shown in parts whose proper connection is indicated by broken lines.)

5 to 125 μ (mostly at intervals of 10 to 50 μ); adhesive protuberances sessile, obovoid or prolate ellipsoid, mostly 6 to 9.5 μ long, 2 to 3 μ wide at the base, measuring 3.8 to 5.5 μ in greatest width, usually filled at the base with homogeneous contents but in the distal region filled with granular protoplasm, often holding fast to nematodes, then individually perforating the integument of the adhering animal and intruding a globose infective body from which assimilative hyphae are extended to appropriate the fleshy contents; assimilative hyphae eventually divided into segments often 15 to 25 μ long and 2 to 4.5 μ wide; conidiophores colorless, erect, often containing 3 to 9 cross-walls, commonly 250 to 325 μ high, 3.5 to 4.5 μ wide at the base, tapering gradually upward, about 2 μ wide at the tip on which a single conidium is borne; conidia colorless, usually spindle-shaped, truncate and rather narrow at the base, rounded at the distal end, mostly 40 to 60 μ (average about 49.2 μ) long, 11 to 18 μ (average about 14.4μ) wide, commonly divided by 4 cross-walls into 5 cells whereof the one in middle position usually exceeds the others in length and width.

Capturing and consuming various nematodes (*Aphelenchoides subtenuis* among them) it occurs in decaying plant materials near Laplace, Louisiana.

Dactylella phymatopaga was first discovered as it developed sparingly in two maizemeal-agar plate cultures that had been planted with decaying vegetable material collected in southern Louisiana on December 20. 1952. In both cultures it appeared somewhat tardily, after several other clampless predacious hyphomycetes, including the new *Arthrobotrys anchonia* and the knob-bearing *Dactylaria haptospora,* had operated extensively in the destruction of eelworms. The admixture of *Dactylaria haptospora,* fortunately, added no special difficulty since the stalked adhesive knobs of that species were very readily distinguishable from the sessile knobs (FIG. 5, *A, a–j; B, a–c; C, a–c; D, a–c; E, a–d; F, a–e; G, a–c; H, a; I, a; J, a, b; K, a; L, a.* FIG. 6, *A, a, b; B, a, b; C, a–c; D, a, b*) borne laterally on the mycelial hyphae of the present fungus. When occasion offered, these sessile adhesive organs (FIG. 5, *G, d; H, b*) functioned successfully in the capture of motile nematodes, most of the animals taken belonging to a slender species which Dr. G. Steiner determined as probably being *Aphelenchoides subtenuis* Cobb. Penetration of the captive's integument, invasion of its interior (FIG. 5, *G, d; H, b*), and expropriation of its fleshy substance took place much as in related forms. When all living contents had been withdrawn from the assimilative hyphae their empty membranes soon vanished, but the membranous envelopes of the infective body and the adhesive cell (FIG. 5, *I, b, c*) often became thickened and then remained

visible long after movement of protoplasm backward into the parent hypha was completed.

The conidial apparatus produced by *Dactylella phymatopaga* in the nematode-infested cultures appeared of relatively uniform character. Each conidiophore in its erect posture (FIG. 5, *J, c; K, b*) formed a single conidium (FIG. 5, *J, d; K, c*) on its tip. Often it would then fall over on to the moist substratum, with some of its proximal segments still filled with protoplasm. From one of these living segments (FIG. 5, *L, b*) would now be sent up a new conidiophore (FIG. 5, *L, c*) that would bear a new conidium. The same sequence of events could then be repeated again and again with resultant development of additional spores.

The conidia of *Dactylella phymatopaga* (FIG. 5, *M, a–u*) show general parallelism in outward shape and cellular make-up to those of *Dactylella ellipsospora* Grove (**3**: 492–496), *Dactylaria haptotyla* Drechsl. (**8**: 41–49), and *Dactylaria sclerohypha* Drechsl. (**8**: 49–58) among the several species employing stalked adhesive knobs in the capture of nematodes. After falling on a stale substratum they often give rise, more especially from the parabasal cell (FIG. 5, *N*), to a slender aerial outgrowth which frequently develops into a conidiophore and bears a secondary conidium. Through repetition of the process conidia of successively higher orders may be formed, each somewhat smaller than the parent it replaces, though in all observed instances the reduction in size was not pronounced. Measurements on 25 quadriseptate conidia selected at random, and therefore very probably including some individuals of the secondary and tertiary orders, were used to obtain the averages for the main conidial dimensions given in the diagnosis. The 25 measurements for total length and for greatest width ranged from 41.3 to 58.0 μ and from 11.2 to 17.3 μ, respectively, while supplementary measurements for lengths of the 5 component cells gave ranges and averages as follows: basal cell, 6.8 to 12.4 μ, 9.3 μ; parabasal cell, 5 to 9.7 μ, 7.6 μ; middle cell, 14.7 to 25.5 μ, 19.1 μ; penultimate cell, 4.1 to 7.6 μ, 5.8 μ; apical cell, 5.3 to 11.8 μ, 7.4 μ.

If conidia of *Dactylella phymatopaga* fall on moist fresh agar they usually germinate by extending one or more germ hyphae on or into the substratum. These procumbent or submerged germ hyphae sometimes originate near the proximal end of the basal cell (FIG. 5, *O, P*). In the presence of motile eelworms they soon give rise to sessile adhesive knobs (FIG. 5, *Q, a–m*), and are then ready to subsist predatorily. Predacious organs, however, are not usually formed where no alien organisms are present, and therefore are not generally produced

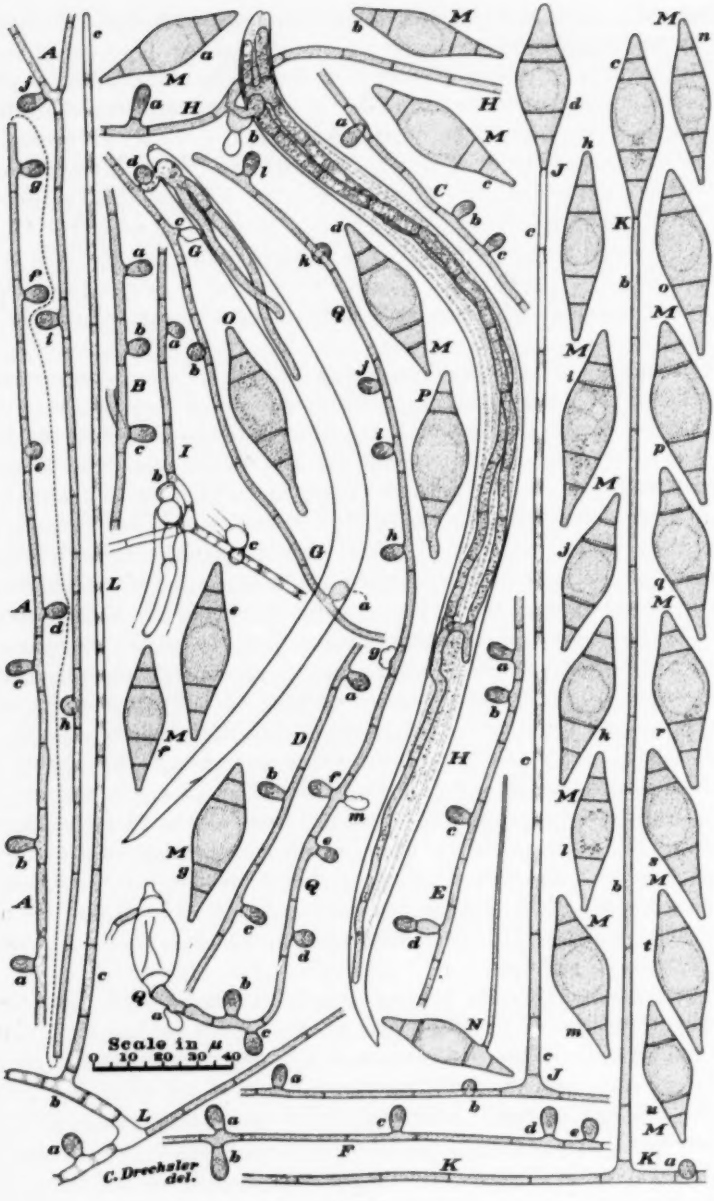
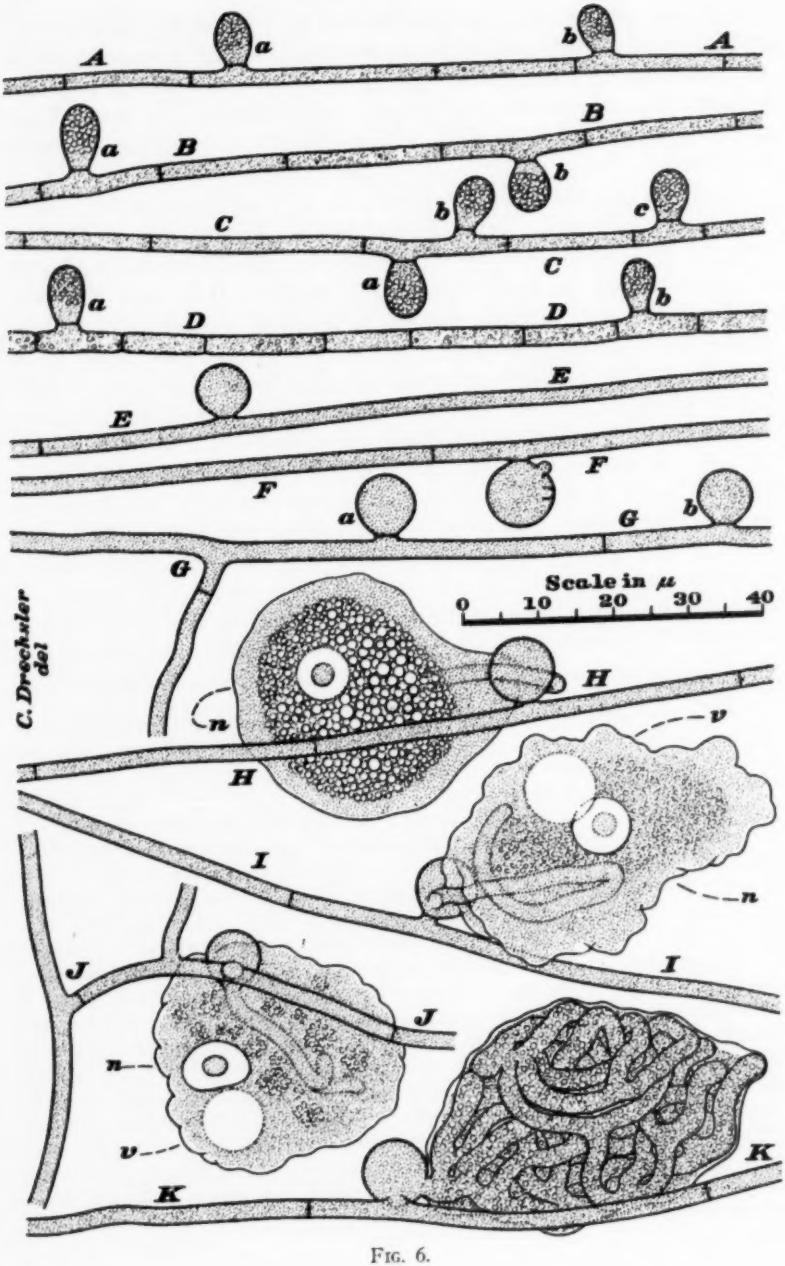


FIG. 5.

in the pure cultures often readily obtained by transferring conidia from the erect conidiophores to tubes of maizemeal agar, if contamination is avoided throughout use of sterile agar slabs held on a flamed platinum spatula. In such cultures the fungus usually produces no conidial apparatus, but, instead, often forms rather extensive wefts of cottony aerial mycelium.

Dactylella phymatopaga is recognizable as a separate species mainly by its adhesive knobs. These knobs (FIG. 6, *A*, *a*, *b*; *B*, *a*, *b*; *C*, *a–c*; *D*, *a*, *b*) are distinguished from the similarly unicellular adhesive knobs of several other nematode-capturing hyphomycetes in that they are sessile rather than stalked, obovoid rather than globose, and contain granuliferous protoplasm at the distal end rather than being filled throughout with relatively homogeneous protoplasm. In respect to their lack of stalks they show parallelism not only with the digitate protuberances employed by the related *Pedilospora dactylopaga* Drechsl. (**1**) in capturing testaceous rhizopods, but also with the globose protuberances used by my *Dactylella tylopaga* (**2**) in capturing *Amoeba verrucosa* Ehrenb. The protuberances of *P. dactylopaga*, whether formed on the mycelial hyphae or on detached conidia, are, however, much smaller than the adhesive knobs of the present fungus, and differ, besides, in not being delimited from the parent cell by a cross-wall. It is not certain that the protuberances of *D. tylopaga* are likewise not delimited at the base, as only a few of these organs occurred in the material from

FIG. 5. *Dactylella phymatopaga* as found in nematode-infested maizemeal agar culture; × 500 throughout. *A*, Portion of mycelium with 10 sessile adhesive knobs, *a–j*. *B–D*, Portions of hyphae, each with 3 sessile adhesive knobs, *a–c*. *E*, Portion of hypha with 4 adhesive knobs, *a–d*. *F*, Portion of hypha with 5 adhesive knobs, *e–e*. *G*, Portion of hypha with 4 adhesive knobs, *a–d*; one knob (*c*) has become emptied of protoplasm, and another (*d*) has served in capturing and invading an eelworm probably of the species *Aphelenchoides subtenuis*. *H*, Portion of hypha with 2 adhesive knobs, *a* and *b*; one knob (*b*) has served in capturing an eelworm (probably *Aphelenchoides subtenuis*) which now is invaded from head to tail. *I*, Portion of mycelium with 3 sessile knobs, *a–c*; two of the knobs (*b*, *c*) have each served in capturing an eelworm and are now being emptied of their protoplasm. *J*, Portion of hypha with 2 adhesive knobs, *a* and *b*, and a conidiophore, *c*, bearing a single conidium, *d*. *K*, Portion of hypha with one adhesive knob, *a*, and a conidiophore, *b*, bearing a single conidium *c*. *L*, Portion of hypha with an adhesive knob, *a*, and with the proximal portion of a fallen conidiophore, *b*, that has sent up a new conidiophore, *c*. *M*, Random assortment of 21 conidia, *a–u*, showing usual variation in size, shape, and cellular dimensions. *N*, Detached conidium with erect filamentous outgrowth. *O*, *P*, Two conidia, each with short vegetative germ tube. *Q*, Empty conidium with germ hypha bearing 13 adhesive knobs, *a–m*. (Owing to lack of space *A* is shown in 2 parts whose proper connection is indicated by broken lines.)


A
a
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Scale in μ
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FIG. 6.

which the species was originally described, and no other relevant material has since come under observation.

Another fungus with sessile adhesive knobs recently came to light in a maizemeal-agar plate culture that had been planted with leaf mold kindly collected by W. F. Jeffers on March 7, 1953, near Willards, Maryland. In this fungus the knobs (FIG. 6, *E; F; G, a, b*) were of subspherical shape and measured 7 to 9.5 μ in diameter. They were borne at longish intervals on clampless, sparingly septate hyphae, mostly 1.8 to 2.8 μ in width. Like the parent hyphae, from which they were delimited individually by a basal septum often about 2 μ wide, they were filled throughout with protoplasm of homogeneous appearance. They served effectively in capturing a species of *Amoeba* usually about 35 μ wide when drawn into a somewhat rounded form (FIG. 6, *H–K*). Attack on the adhering animal began with the intrusion of a single hypha. During the earlier stages of invasion the protoplasm of the captive underwent no marked change in texture, its contractible vacuole (FIG. 6, *I, v; J, v*) continued to operate regularly, and its nucleus—a globose body (FIG. 6, *H–J; n*) measuring commonly 7 to 8 μ in diameter and containing a slightly darker endosome 2.5 to 3 μ wide—showed no abnormality. In later stages the animal's internal structure was gradually obliterated as the simple infective hypha first intruded gave rise to an intricate tangle of assimilative branches (FIG. 6, *K*). Although the predacious mycelium would seem to have obtained ample nourishment no reproductive bodies were formed. The fungus, in any case, appears noteworthy especially because of the similarity of its predacious organs to those of *Dactylella phymatopaga*.

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FIG. 6. Drawn at a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A–D*, Portions of hyphae of *Dactylella phymatopaga* from nematode-infested maizemeal agar, each with 2 (*A, B, D*) or 3 (*C*) sessile adhesive knobs. *E, F*, Portions of hyphae of unnamed *Amoeba*-capturing fungus, each with a sessile adhesive knob; the knob in *F* shows 2 protuberances which presumably represent incipient infection hyphae that were put forth while an *Amoeba* was temporarily being held captive. *G*, Portion of mycelium of same *Amoeba*-capturing fungus with 2 adhesive knobs, *a* and *b*. *H–J*, Portions of mycelium of same fungus, each with an adhesive knob from which an assimilative hypha has been extended into a captured *Amoeba; n*, nucleus of animal; *v*, contractile vacuole of animal, shown in *I* and *J* but not in *H*. *K*, Portion of hypha of same fungus, with an adhesive knob from which an intricate tangle of assimilative branches has invaded a captured *Amoeba*.

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TAXONOMIC NOTES ON TUBERALES

HELEN M. GILKEY

(WITH 14 FIGURES)

Examination, during the past several years, of many collections of hypogaeous ascomycetes, and the privilege of spending two months at the New York Botanical Garden with its specialists and its unexcelled library and herbarium facilities, have resulted in recognition by the writer of three new genera, six new species, and the need of modifying certain earlier concepts concerning the status of previously published forms.

In line with these studies, a revision of the Tuberales has been prepared for the North American Flora; and in this revision are included the new American taxa. The present paper presents an Australian genus and a species, previously undescribed, together with miscellaneous observations not appropriate to the scope of the larger work. The accompanying plate illustrates new species described in both papers.

The weeks of study in New York were made possible by three grants, one each from the American Philosophical Society, the Mycological Society of America, and the General Research Fund at Oregon State College. For all these I am grateful, for the experience afforded the opportunity of a broader insight into the taxonomy of the order, and provided a basis for the conclusions here expressed. It must be understood, however, that until complete life histories in this group are better known, unanswered questions will still remain concerning accurate boundaries of the order itself, as well as true relationships within it.

My thanks go to many persons. Particularly I wish to mention Dr. D. P. Rogers, curator of the mycological herbarium at the New York Botanical Garden, for initial encouragement toward preparation of the two papers, and for unfailing help in many times of need.

A NEW GENUS FROM AUSTRALIA

Hydnoplicata gen. nov.

Ascomatibus lobatis, puncto adjunctionis myceliali destitutis; gleba plicis compactis et irregularibus, nonnumquam anastomosantibus, et fossas longas labyrinthiformesque et cubicula separantibus composita, ascis et paraphysibus in vallum ordi-

natis vestita; ascis cylindraceis; paraphysibus quam asci longioribus, apicibus in pseudoparenchyma intertextis; sporis uniseriatis, breve ellipsoideis.

Ascocarp lobed, without basal attachment, with internal folds forming canals and chambers opening at several points to the surface and lined by a palisade of asci and paraphyses; asci cylindrical; paraphyses fused above asci to form a more or less pseudoparenchymatous epithecium; spores smooth, ellipsoid, uniseriate.

The fungus which, in lobing, internal folding, and the presence of several external openings, suggests certain species of *Hydnotrya,* differs from that genus in the possession of an epithecium and in smooth spores. In all species of *Hydnotrya* thus far described, the ascocarp surface is minutely villose, the villi being transformed at the openings into free paraphyses. In *Hydnoplicata,* on the contrary, the surface is glabrous; none of the paraphyses are free but are fused above the asci in a casual pseudoparenchyma. Such an epithecium has previously been known in the Tuberales only in the *Petchiomyces-Genea-Myrmecocystis-Genabea* line. From the last three genera, *Hydnoplicata* is distinguished by its very slender asci, non-sculptured spores, and structure of the sterile context; while it differs from *Petchiomyces* in its complexly folded ascocarp and absence of mycelial tuft.

Type species, *Hydnoplicata Whitei* Gilkey.

Hydnoplicata Whitei sp. nov.

Ascomatibus 1.5–2 cm diam., dilute rubidis, delicatis, levibus; texto corticis pseudoparenchymatico; ascis gracilibus, sporis levibus, 6.5–8 × 10–11 μ.

Ascocarp 1.5 to 2 cm in diam., Seashell Pink (R.), conspicuously lobed and folded, delicate in texture, surface smooth; cells all thin-walled; cortex largely pseudoparenchymatous, the cells widely variable in size; asci slender; spores smooth, ellipsoid, 6.5–8 × 10–11 μ. (FIGS. 13, 14.)

Sydney, Australia, at grass roots, 20 ft. from a *Eucalyptus* grove, N. H. White, *type* (1/2 in Dr. White's collection, Sydney, Australia; 1/2 no. *540,* H.M.G.).

With appreciation this new fungus is named for Dr. N. H. White, Senior Lecturer in Plant Pathology, University of Sydney, Australia; not only for his part as discoverer, but also for his continuing efforts to acquaint the world with the Tuberales of his continent. Of the five previous reports from Australia and Tasmania, two names, as indicated elsewhere in this paper, are found to be *nomina confusa* based on immature specimens plus a parasite, the status of at least one of the others

is uncertain (Fischer, **1938**), while the location of the two remaining collections is unknown to me. We know, however, from even these feeble reports, that these fungi do exist on the continent "down under"; and this is verified by Dr. White in at least two instances.

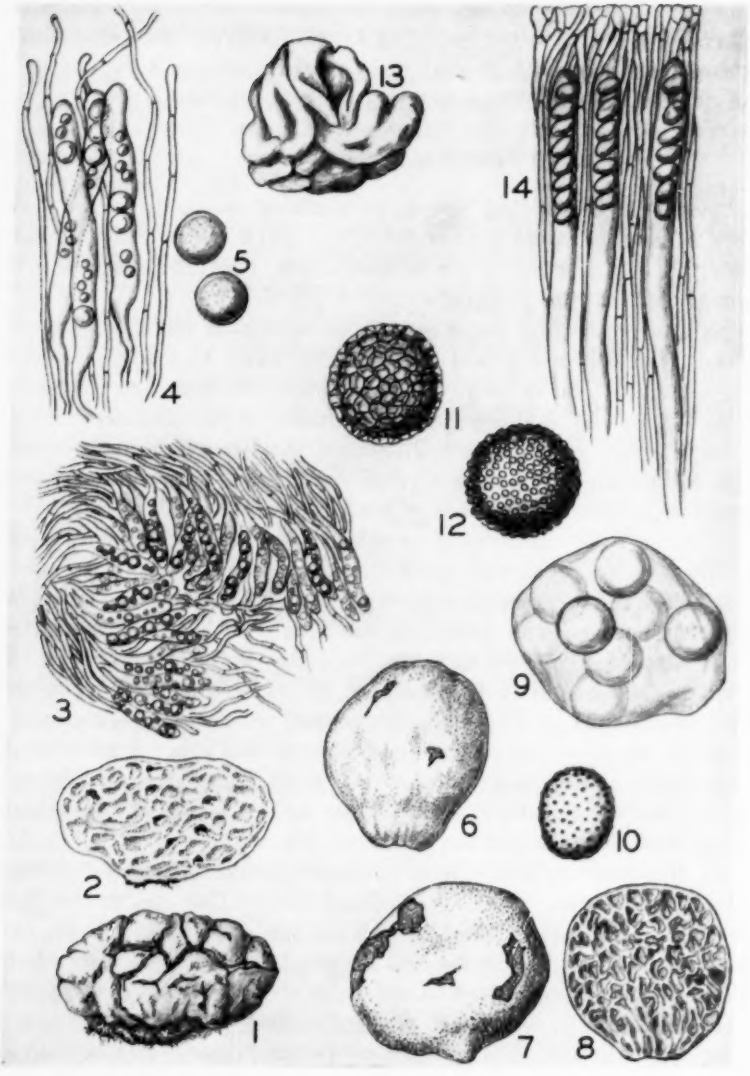
TWO NOMINA CONFUSA

Among the mycological collections available for study at the New York Botanical Garden Herbarium is that of Dr. George Massee; and during its examination by the present writer, two interesting cases of *nomina confusa* came to light.

On a sheet bearing the notation: "*Leucangium Readeri* Cke. and Mass., *type*, Victoria (Dimboola) Reader, damp clay soil," occurs a single specimen, cut at one end apparently for sectioning, and now measuring 0.5 cm. With it are drawings, in color, of four ascocarps, also of a single spore; and, in pencil, a rounded ascus containing four spores, with several separate spores to show variation in shape. The spores, though somewhat variable, are in general lemon-shaped.

A microscopic examination reveals the fact that the fungus is not *Leucangium* (a genus now generally regarded as a section of *Picoa*); for though the ascocarp is immature, hyaline globose spores in cylindrical asci arranged in a palisade can faintly be seen, a hymenial arrangement which differs decidedly from that of the latter genus. In addition, however, dark lemon-shaped spores resembling those figured by Cooke and Massee are borne abundantly in spherical perithecia of a parasitic fungus which crowds and distorts the host. I have as yet been unable to determine whether this "new species" was actually published; but the drawings accompanying the "type" illustrate ascocarps of the host and spores of the parasite.

In the same collection is a specimen labeled "*Genabea tasmanica* Mass. and Rodw., Tasmania, Rodway *119*." This species was published in Kew Bull. **1898**: 125, with a Latin description and the comment in English, "a very fine and distinct species * * * remarkable for the very large lemon-shaped spores which are quite smooth, clear brown, and translucent." Dr. D. P. Rogers confirmed my suspicion that in this, also, the fruiting body belonged to one fungus, the spores to another. At his suggestion, both cases were referred to Dr. W. W. Diehl of the Bureau of Plant Industry, who identified the parasite in each as *Melanospora Zobelii* (Corda) Fckl. Since no asci or spores belonging to the host were sufficiently mature for determination, its identity must remain unknown.



FIGS. 1–14. *Densocarpa Shanori*. 1. Exterior of ascocarp. 2. Longitudinal section of ascocarp. 3. Portion of hymenium at end of a cavity. 4. Section of hymenium showing asci and paraphyses. 5. Spores. 6–9. *Carbomyces emergens*. 6, 7. External views of ascocarps, slightly damaged by insects. 8. Longitudinal section, showing slight elongation of veins at base. 9. Ascus containing spores. 10.

GENEA—MYRMECOCYSTIS—GENABEA LINE

In any key or phyletic chart of the Tuberales a conspicuous offshoot from the main developmental line may be found taking off early in the history of the order, or perhaps from its predecessors, developing through a few genera or subgenera to a high degree of complexity and sterilization, then ending abruptly. This is the *Genea-Genabea* series to which Harkness (**1899**) added *Myrmecocystis* [*Pseudogenea* Bucholtz (in Mattirolo, **1900**); *Genea* subgenus *Myremecocystis* Gilkey (**1916**)].

The ascocarp of *Genea* in its simplest form (section *Eugenea*) is a hollow ball with a single opening at the apex and a mycelial tuft at the base. Except for its heavily sculptured spores and its cortex-like epithecium, it considerably resembles a regular or sometimes slightly lobed ascocarp of a young *Peziza*. Between the outer cortex and the epithecium the hymenium is continuous, and the spores are ellipsoid.

In the section *Heterogenea*, a mycelial tuft is still present; but external lobing, with concurrent development of internal folds and projections from the ascocarp wall, becomes conspicuous. This modification is accompanied by considerable sterilization of the hymenium, resulting in fertile "pockets" separated by occasional sterile strands. The spores are broadly ellipsoid to subglobose.

The genus *Myrmecocystis*, as represented by the type species, *M. cerebriformis* Hark. (**1899**), is characterized by absence of a mycelial tuft, by extreme lobing and folding, by generally several openings from the hymenium, conspicuous sterilization of hymenial areas, crowded asci and paraphyses, and globose spores. With the discovery, however, that *Hydnocystis compacta* Hark. is not *Hydnocystis* but a clear-cut member of the *Genea* complex, the present writer in an earlier publication (Gilkey, **1916**) reduced *Myrmecocystis* to subgeneric status under *Genea*. *Hydnocystis compacta* Hark. became *Genea intermedia* Gilkey (the specific epithet, *compacta*, having been preempted in *Genea*), on the ground that it represented an intermediate form, combining the simple cup-like structure and single opening of *Eugenea* with the sterile areas, crowded asci, globose spores, and absence of mycelial tuft of *Myrmecocystis*.

The publication of *M. vallisumbrosae* (Bucholtz) Fischer (**1908**), a European species likewise simple in form, helped to confirm the above concept of the genus. But while this arrangement disposed of certain difficulties encountered in the complex, it did not, as the writer recog-

Carbomyces Longii, single spore. 11. *Tuber Lindsalei*, single spore. 12. *Choiromyces Cookei*, single spore. 13, 14. *Hydnoplicata Whitei*. 13. Ascocarp. 14. Portion of hymenium showing epithecium.

nized, provide for the obvious relationship to *Genabea* which had accomplished a further step in sterilization, resulting in fertile areas isolated from each other by pseudoparenchyma. Its two recognized species, *G. fragilis* Tul. and *G. sphaerospora* Matt., have respectively ellipsoid and globose spores. (*G. tasmanica* Massee and Rodway is shown elsewhere in this paper to be a *nomen confusum.*)

Although the extremes of this complex, *Genea* and *Genabea,* are clearly distinguishable, the intermediate steps are so gradual that only two logical courses are open: either to telescope the entire line in *Genea,* or recognize both *Myrmecocystis* and *Genabea* as genera. The latter course, which was followed by Fischer (**1938**, p. 15), appears preferable, though it still must be evident that the three genera bear closer relationship to each other than to any other genus known in the order.

The discovery in recent years of a hypogaeous species of *Petchiomyces* (Gilkey, **1939**, p. 15), and description in the present paper of a new genus, *Hydnoplicata,* somewhat complicate this otherwise well-defined line; for although both these genera also exhibit a more or less conspicuous epithecium, neither appears to take a logical place in the succession. Whether, however, they occur as offshoots from the main line, or as of independent origin, they may reasonably be placed at present in Geneaceae, and keyed as follows:

Mycelial tuft present; opening to hymenium single; spores ellipsoid
  Spores smooth or minutely granular ..... *Petchiomyces*
  Spores sculptured ..... *Genea*
Mycelial tuft absent
  Spores sculptured, ellipsoid or globose
    Hymenium interrupted by sterile strands, but not partitioned into isolated areas separated by pseudoparenchyma; opening one or several; spores globose ..... *Myrmecocystis*
    Hymenium in small isolated areas separated by pseudoparenchyma; openings several; spores ellipsoid or globose ..... *Genabea*
  Spores smooth, ellipsoid ..... *Hydnoplicata*

With the restoration of *Myrmecocystis, Genea intermedia* becomes *Myrmecocystis compacta* (Hark.) Gilkey.

PSEUDOBALSAMIA MICROSPORA DIEHL AND LAMBERT

A long overdue transfer of *Pseudobalsamia microspora* Diehl and Lambert from the Tuberales to the Eurotiales is, at the request of Dr. Diehl, here proposed.

The above name was published (Diehl & Lambert, **1920**) for a newly reported pest in mushroom beds. The fungus was attracting consider-

able attention by its abundant production of mycelium, not only within the compost of commercial beds, but often also as a dense blanket covering the surface. In this mycelial mass, ascocarps of irregular form and varying in size from one to 3 or more cm were found, developing from concentrations of tangled hyphae, sometimes singly but often by the fusion of several such knots. This exceeding variability in size, shape, and origin, together with the fact that such ascocarps as matured underground were generally formed within air spaces of the substratum, conforming to their size and shape rather than following a predetermined pattern of development, undoubtedly explained the internal irregularity which simulated venae externae and internae.

The indecisive character of the ascocarp, the peridium consisting wholly of interwoven hyphae, and the internal disintegration at maturity resulting in a powdery residue of spores combine to suggest relationship to the Gymnoascaceae of the Eurotiales. Since *Pseudobalsamia* is a recognized genus of the Tuberales, and since no present genus of the Eurotiales appears appropriate to the organism, I propose the name *Diehliomyces* in honor of Dr. W. W. Diehl, who carefully worked out the life history of the fungus as first described and as far as it is still known. I take special pleasure in so naming the genus, since I was in large part responsible for its earlier incorrect classification.

The generic and specific diagnoses which follow are compiled largely from the original descriptions by Diehl and Lambert, with additional observations from specimens kindly supplied by Dr. Diehl.

Diehliomyces gen. nov.

Ascomatibus hypogaeis vel epigaeis, a mycelio denso antecessis, e hypharum nodis efformatis, difformibus, connatis; ascis inter ascomatum hyphas stipatis, in plicis fertilibus a hyphis sterilibus separatis; sporis 8 vel paucioribus, haud regulariter ordinatis, ut pulvis luteus ab ascarum dissolutione liberatis.

Fungus hypogaeous or epigaeous, characterized at first by a dense mycelial growth in or on the substratum; ascocarps casually developed, without definite form or pattern, from tangled knots of hyphae, several small irregular knots sometimes coalescing into a single large ascocarp; asci closely packed among large interwoven hyphae of fruiting folds, these folds separated by loosely arranged hyphae of sterile areas; spores 8 or fewer, irregularly arranged, released by disintegration of asci and hyphae as a yellow powdery mass.

Type species, *Pseudobalsamia microspora* Diehl and Lambert.

Diehliomyces microsporus (Diehl and Lambert) comb. nov.
Pseudobalsamia microspora Diehl and Lambert, Mycologia **22**: 223. *pl. 27.* 1930.

Ascocarp hypogaeous or epigaeous, irregularly formed, subspherical to discoid, lobed and wrinkled, cream-colored to reddish brown, 1–3 cm in diam., fleshy, consisting of interwoven hyphae with masses of embedded asci; asci fugacious, ovoid to subspherical, short- or long-stipitate, 12–15 × 18–25 μ, with 8 or fewer spores; spores globose or nearly so, smooth, hyaline, 5–7 μ, remaining as a sulphur-colored mass after disintegration of asci and hyphae; chlamydospores occasionally seen in hyphal web of ascocarp, globose, 13 μ, smooth or minutely granular, with golden brown center, epispore 2 μ thick.

Known only in mushroom beds, within compost or on surface.

New York: Rosendale.

Pennsylvania: Concordville.

Ohio: Ashtabula, Dallas Luce and E. B. Lambert, *type* (Mycological Collections, Bur. Pl. Ind.).

Minnesota: Minneapolis.

HYDNOTRYA CUBISPORA

A fungus which later proved to belong to the *Hydnotrya* complex and was so named (Gilkey, **1939**, p. 23) was first described under the name *Genea cubispora* Bessey and Thompson (**1920**). Its identification with the latter genus was suggested to its discoverers by the simple cup-like ascocarp and the elongated paraphyses, which upon later study, however, were found simply to overtop the asci, not to fuse above them as in *Genea*. Dr. Ed. Fischer (**1938**, p. 21) suggested the inclusion of this taxon in *Gyrocratera* Hennings, though he noted an absence of subhymenial asci which are somewhat conspicuous in previously described species of this genus.

In a discussion of the two genera (**1927**) and in his later monograph of the family (**1938**, p. 20), Fischer keyed them essentially thus:

Ascocarp hollow with a single large terminal pore, the inner surface simple or thrown up into folds and projections ..... *Gyrocratera*
Ascocarp generally with many chambers or canals, and with generally several openings from the hymenium ..... *Hydnotrya*

In both genera he noted sculptured spores and the presence of subhymenial asci.

As mentioned earlier (Gilkey, **1947**), discovery of several new American species weakens the main distinction established between the two

genera, a distinction which Fischer himself recognized as faulty. In a long series of collections by Dr. W. B. Cooke on Mt. Shasta, two extremes were found in the ascocarp of *Hydnotrya variiformis* Gilkey, from a simple *Peziza*-like or *Gyrocratera*-like form with a single opening, to a structure extremely convoluted externally, partitioned internally, and with several openings. Indeed, variability in growth form in this species extended even, in a few cases, to an actual and almost complete turning inside-out of the main body of the ascocarp, carrying the hymenium to the exterior surface, as in *Sphaerosoma*.

That no good case, therefore, can be made for splitting *Hydnotrya* into more than one genus, on the basis of complexity, seems clear. This view is further strengthened by a series of specimens of undoubted *H. cubispora* recently received from Dr. K. A. Harrison of the Department of Agriculture of Canada. The series extends from very young to mature ascocarps, at least one of the latter conspicuously partitioned into cavities, with several external openings. With the breaking down of this principal barrier between the two genera, there seems no justifiable reason for retaining *Gyrocratera;* and *Hydnotrya cubispora* is retained by the present writer as the legitimate name for the species in question.

Dr. Harrison's collections and others have necessitated a revision of spore description in *H. cubispora*. Much of the material previously seen in this species is young; but with access to older specimens, we find that, contrary to our earlier concept, the cubical nature of the spore often remains through maturity, and the basic shape is ellipsoid (sometimes very short) rather than globose. The conspicuous thickening which occurs in the epispore frequently is exceedingly asymmetrical, resulting for the most part not in papillae, as previously illustrated, but often in irregular slab-like applications to the original spore. These may occur at the ends or the sides, the terminal spore often by such thickening becoming almost grotesquely elongated.

HYDNOTRYA TULASNEI, H. CARNEA, AND H. INTERMEDIA

The first recognized species of this genus was published as *Hydnobolites Tulasnei* Berkeley and Broome (**1844**), but later (**1846**) transferred by the same authors to their newly-established genus *Hydnotrya*. Shortly afterward, *H. carnea* (Corda) Zobel was published (in Corda **1854**), and for many years the two species have figured in a confused nomenclature. They are now generally recognized as two extremes of an unstable complex, *H. Tulasnei* characterized by clavate asci, irregularly placed spores, and abundant and conspicuous subhymenial hyphae;

while *H. carnea* is known by cylindrical asci, uniseriate spores, and few subhymenial asci. Confusion has resulted, however, from the fact that intermediate stages are common, Bucholtz (**1904**) accordingly establishing the subspecies *intermedia* of *H. Tulasnei* to accommodate these. Soehner (**1942**) later gave this taxon specific rank.

Whether we recognize three species in this complex, or three subspecies or varieties—*typica, intermedia,* and *carnea*—of *H. Tulasnei,* or *H. Tulasnei* and *H. carnea* as species, with *intermedia* as a variety or subspecies of one or the other (all have been suggested as solutions), we still are faced with the fact that they are much more closely related to each other than to any other known species. In America, five endemic species occur, all distinctly different from each other and from those of Europe. But representatives also of the *H. Tulasnei-intermedia-carnea* complex have now been discovered in Pennsylvania, Maine, Michigan and New York; and, as in Europe, they are confusing.

A long series of these, numbering a hundred or more collections and several hundred specimens, was taken by Dr. Alexander Smith in Tahquamenon State Park, Michigan, in 1951, furnishing an excellent opportunity for studying all stages in the ascocarp. For comparison, not only were the European collections in the New York Botanical Garden herbarium available, but specimens and data were received directly through the kindness of Dr. Ert Soehner and others. Also, through the kind offices of Dr. Rogers, mycological curator at the New York Botanical Garden, and the generosity of Sir Edward Salisbury, Director of Kew Gardens, portions of ascocarps from the Berkeley herbarium were received for study. Whether or not the latter were of the type collection, was not indicated; but Dr. Salisbury stated that they probably were supplied by Broome and were collected at Spye Park, the type locality.

While these specimens represent the *H. Tulasnei* extreme, with principally clavate asci and irregularly arranged spores, yet certain of their asci are narrowly cylindrical with uniseriate spores. In the American material previously known from Pennsylvania and Maine, the asci from mature ascocarps are rather predominately cylindrical, as in *H. carnea;* and this is true also of Dr. Smith's series. However, young asci are almost universally clavate with irregularly placed spores, many of the mature asci are similar in shape and spore arrangement, and subhymenial asci are frequently abundant. Many of our forms perhaps most nearly approach Bucholtz's *H. intermedia,* but every gradation is found, together with the two extremes; and it is impossible to break up the complex into species comparable to our five endemics. Varieties might

perhaps be recognized, but certainly no clear-cut lines of separation exist even for these. Therefore, although this disposition of the species runs counter to that of several European mycologists, who, however, had no opportunity to consider recently reported American species in their earlier concepts, it is the judgment of the present writer that at least all American members of this group may be referred to *H. Tulasnei*, though with full recognition of the perplexing variability.

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TWO SPECIES OF GALZINIA FROM THE SOUTHERN APPALACHIANS

LINDSAY S. OLIVE

(WITH 21 FIGURES)

During the summer of 1953, while the writer was searching for species of jelly fungi, two resupinate mucous-gelatinous specimens resembling somewhat in appearance and texture certain members of the genus *Gloeotulasnella* were included among the collections. When these specimens were examined microscopically it was discovered that they belonged to the genus *Galzinia* Bourdot (**1921**) of the Thelephoraceae (Rogers, **1944**). Bourdot described a single species, *G. pedicellata* Bourd., which has thin, resupinate, mucous fructifications; vesicular probasidia; long basidia with slender intermediate section and expanded apex, showing a distinct demarcation from the probasidia; and two to four sterigmata producing curved, cylindric basidiospores.

Rogers redescribed the type species from collections obtained in Pennsylvania and Oregon and described two new species, *G. cymosa* and *G. occidentalis*. *G. cymosa* is mucous-gelatinous and is distinguished from the other two species on the basis of texture and several microscopic characters such as the absence of clamp connections. *G. occidentalis*, like *G. pedicellata*, possesses clamp connections, but the former species has waxy-pruinose fructifications and large gloeocystidia among its basidia. All three species have been reported only on coniferous wood, mainly pine.

One of the writer's collections is quite obviously a new species, differing conspicuously from the three previously described species in having bifurcate basidiospores. The second specimen is clearly identical with *G. cymosa* and in most details fits very well Rogers' description of it. Since this is the second collection of the species a brief description of it is included in this paper.

Galzinia geminispora sp. nov. (FIGS. 1–13.)

Fructificatio resupinata, indeterminata, mucoso-gelatinosa, tenuis, 50–125 μ crassit. Hyphae enodosae, 1.6–7.7 μ diam.; paraphyses irregulariter ramosae, 1.6–3.6 μ diam., in hymenio sparsae; probasidia aggregata, ovalia vel pyriformia, saepe in cauliculum

basalem producta, 4.5–6.8 × 7.2–12.6 μ; basidia typice ex apice probasidiorum orta, longa graciliaque, plerumque ad apicem inflata, cum probasidio 2.7–6.3 × 19–68 μ, sterigmata quattuor 4.5–8.1 μ long. producentia; basidiosporae e dimidiis geminis apiculum versus conjunctis constatae, dimidio 2.5–3.7 × 7.2–9.9 μ, per hypham e ramo altero ortam germinantes.

Fructification resupinate and indeterminate, very thin, 50–125 μ in section, soft, mucous-gelatinous. Hyphae without clamp connections, 1.6–7.7 μ diam.; scattered, irregularly branched paraphyses present, 1.6–3.6 μ diam.; probasidia produced in clusters on upright branching hyphae, oval to pyriform, the basal portion often narrowed into a stalk-like structure, 4.5–6.8 × 7.2–12.6 μ; basidia usually arising apically, occasionally subapically or laterally from the probasidia, long and slender, typically somewhat swollen apically, measuring (including probasidia and sterigmata) 2.7–6.3 × 19–68 μ, 4-sterigmate, rarely 2-sterigmate; sterigmata 4.5–8.1 μ long; basidiospores apiculate, forked, with twin branches, each half measuring 2.5–3.7 × 7.2–9.9 μ, each spore typically germinating by means of a single germ tube which arises distally from one arm of the spore.

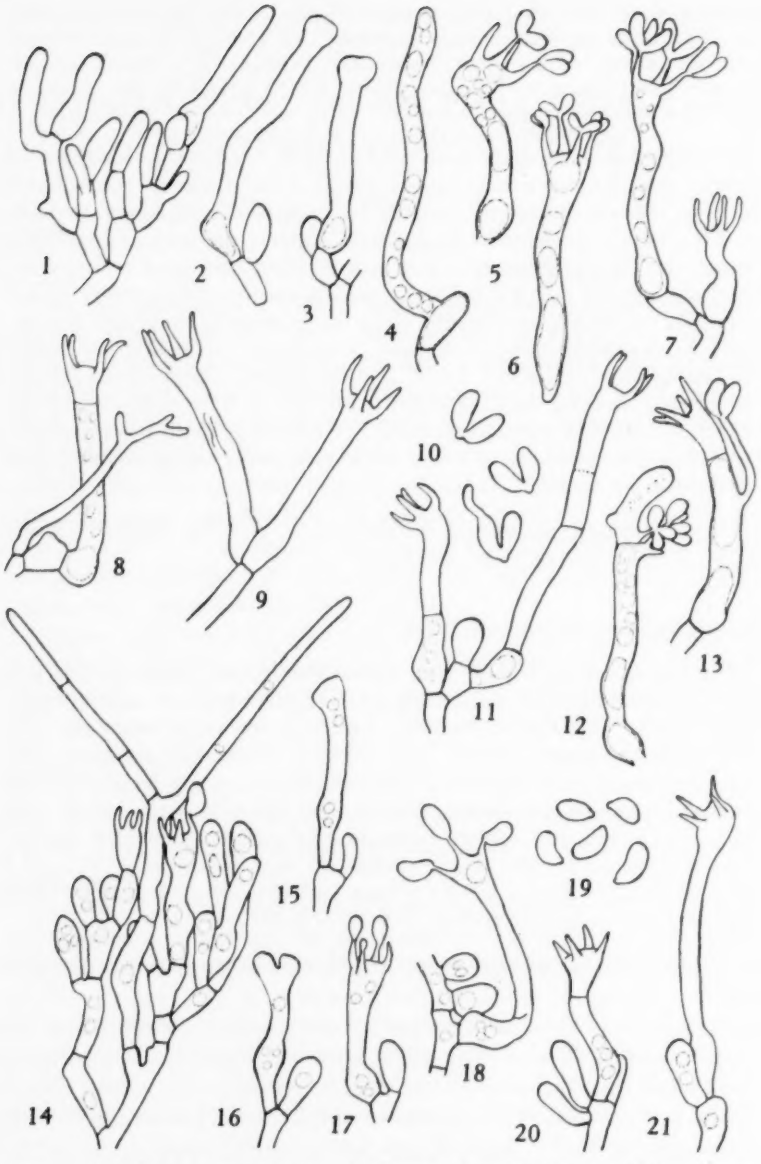
On very rotten, decorticate frondose limb, probably chestnut, Highlands, North Carolina; June 15, 1953.

G. CYMOSA Rogers. (FIGS. 14–21.)

Fructifications resupinate and indeterminate, very thin, 45–145 μ in section, mucous-gelatinous, sordid tan. Hyphae without clamp connections, 1.8–5.4(–6.8) μ in diameter; scattered, sparingly branched, paraphysis-like structures present in hymenium; probasidia clavate to obovate, frequently with narrowed stalk-like base, 4–6.5 × 8–18 μ; basidia frequently, though not always, distinct from the probasidia, usually arising apically from the latter, 4-sterigmate, typically expanded apically, overall dimensions (including probasidia and sterigmata), 4.5–6.8 × 15.3–49.5 μ; sterigmata 3.6–5.4 μ long; basidiospores allantoid to short cylindric and curved, apiculate, 2.7–3.3 × 6.3–7.8 μ.

On very rotten frondose wood, Highlands, North Carolina; June 23, 1953.

Certain features of these fungi deserve further consideration with emphasis upon their possible phylogenetic significance. It has already been made clear by previous workers, as well as in the present descriptions, that the basidia of *Galzinia* typically arise from vesicular cells, called probasidia by Bourdot and Galzin (1927) and by the present writer. There usually remains a distinct demarkation between basidium and probasidium. This demarkation is made more apparent by the



FIGS. 1–13. *Galzinia geminispora*. 1. Cluster of probasidia, two giving rise to basidia. 2, 3. Basidia, showing apical enlargement. 4. Basidium arising laterally from probasidium. 5–7. Basidia producing bilobed basidiospores. 8. Basidium and

occasional tendency, noted here and by previous investigators, of the basidia to arise laterally or sub-apically from the probasidia (FIG. 4). Rogers (**1944**) noted that in one specimen of *G. pedicellata* most of the basidia arose in this manner.

A second feature of unusual interest is the occasional development of a transverse septum (very rarely more than one) across the basidium (FIGS. 8, 11–13) and the rare development of basidiospores laterally on some of these basidia (FIGS. 12, 13). Septum-formation in the basidia probably occurs more frequently in *G. geminispora* than in the other three species, but Rogers illustrates a septate basidium in *G. occidentalis*, Bourdot and Galzin illustrate one in their drawing of *G. pedicellata*, and the present writer has observed them on occasion in *G. cymosa* (FIG. 20).

The fact that species of *Galzinia* are typically mucous-gelatinous in texture and resupinate, that they have persistent probasidia which usually remain distinct from the basidia, and that the basidia are typically long and slender and occasionally septate naturally leads to the conclusion that the genus is a primitive one and that the ancestral type may have been a resupinate member of the Auriculariaceae. The nearest existing relatives might possibly be found in such resupinate genera as *Platygloea* or *Helicogloea*. The writer (**1954**) has recently discussed the probable phylogenetic relationships of these two genera. For example, one would find it difficult up until the time of basidial maturation to distinguish any important differences between *Platygloea arrhytidiae* (Olive, **1951**) and *Galzinia* in probasidial and basidial development. *P. arrhytidiae* produces both 2-celled and 4-celled basidia. It is suggested that the type of basidial development in *Galzinia* may have evolved from a type similar to that found in *P. arrhytidiae* by the loss of the ability to form basidial septa (except on occasion) and the shifting of basidiospore formation to the apex of the basidium. The development of an occasional basidial septum and even of lateral basidiospores in *Galzinia* is considered a recapitulation of the ancestral type of development.

The writer is in agreement with Linder (**1940**) that one of the main

paraphysis; basidium with septum in upper part, the upper cell being empty after discharge of spores. 9. Two empty basidia after spore discharge. 10. Basidiospores, one of which is germinating. 11. Septate basidia. 12. Septate basidium producing basidiospores laterally. 13. Septate basidium showing apical and lateral spore development. FIGS. 14–21. *Galzinia cymosa*. 14. Cluster of basidial elements including branched paraphysis, probasidia, and basidia. 15, 16. Basidia with enlarged apices. 17, 18. Basidia producing basidiospores. 19. Basidiospores. 20. Basidium with septum, the upper cell empty after spore discharge. 21. Empty basidium following spore discharge. (All figures × 942.)

lines of evolution leading to basidiomycetes with the *Corticium*-type basidium and probably to many of the higher basidiomycetes may have had its origin in a *Sebacina*-like ancestor. The writer also agrees tentatively with Linder's theory that the *Tulasnella*-type basidium had its origin in the *Sebacina* type. However, the present findings indicate that species with the urnigera type of basidium, characteristic of the resupinate genus *Trechispora* and others, may not have evolved from a sebacinoid type, as Linder suggests, but from the *Galzinia* type which in turn arose from a *Platygloea*-like form. No species of the Tremellaceae is known to have probasidia which remain distinct from the basidia as is generally the case in *Galzinia* and *Trechispora*. A further indication of the relationship between *Galzinia* and *Trechispora* and of their primitive nature may be found in the fact that gloeocystidia occur in both genera. Furthermore, certain species of *Trechispora* have waxy or waxy-mucedinoid fructifications.

The above statements make it obvious that the present writer is suggesting a polyphyletic origin of the homobasidiomycetes from the heterobasidiomycetes. The line of development leading from such a primitive genus as *Galzinia* may be an extensive one, if the urnigera-type basidium is considered a primary criterion of relationship. The writer is inclined to agree with Rogers (**1944**) in considering it so. Rogers states that ". . . the urnigera basidium and the other characters regularly associated with it are a reliable indication of kinship, and the variations in the hymenial configuration . . . are comparatively trivial and recent variations in a single original type. On this hypothesis it is the urnigera members of the Thelephoraceae, Hydnaceae, and Polyporaceae, that are descended from one common ancestral form or group, and variation in hymenial configuration that has brought about accidental convergence." The present writer suggests that *Galzinia* is the most primitive genus of homobasidiomycetes in this line of evolution.

In passing, it should be noted that basidial development resembling in certain respects that described in *Galzinia* has also been reported in such unusual genera as *Dicellomyces* of the Dacrymycetaceae as well as in *Vuilleminia* and *Brachybasidium*, whose proper position in the homobasidiomycetes is uncertain. The occasional 2-spored basidia found in *Galzinia* resemble quite closely the typical 2-spored basidia of *Dicellomyces* (Olive, **1945**). In *Vuilleminia*, the apex of the basidium is inflated as in *Galzinia*. There is insufficient information at this time to permit a discussion as to whether such forms evolved from the *Galzinia* line or from some other phylogenetic series.

The writer is grateful to Dr. D. P. Rogers for preparing the Latin

description of the new species. Type material of *Galzinia geminispora* has been deposited at the New York Botanical Garden.

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VOLUTELLA SPECIES ON ALFALFA [1]

JOHN E. CHILTON [2]

(WITH 8 FIGURES)

During the winter 1949–50, observations of forage legumes of foreign origin, grown in the Primary Plant Introduction Station greenhouse at Ames, Iowa, revealed a fungus with pink-colored, dark-setose, erumpent fruiting bodies parasitizing stems and leaves. Relationships with *Colletotrichum* were evident, but subsequent study showed the fruiting bodies to be sporodochia, which, with the single-celled conidia and the presence of setae, caused the fungus to be referred to the genus *Volutella*. Subsequently, two other species of *Volutella* were collected, both having prominent sporodochia and hyaline setae, and were identified as *Volutella gilva* (Pers. ex Fr.) Sacc. and *V. ciliata* (Alb. & Schw.) Fr. respectively. Comparative studies of these three species were made and the results are here reported.

Of the four species of *Volutella* that have been reported on forage legumes in the literature, *Volutella flexuosa* Cke. & Ell. with large conidia (18–20 μ) from red clover (**1**) and *Volutella indica* (Niessl.) Sacc. from legume pods (**5**), with long flexuous setae, were not encountered. Of the other two, *V. gilva* (Pers. ex Fr.) Sacc. and *V. ciliata* (Alb. & Schw.) Fr., the first was reported on alfalfa and sweet clover in Europe (**4**) and later as a saprophyte on alfalfa by Stewart *et al* (**7**) from the United States. The second was listed from alfalfa in Europe by Lind (**4**) as well as by Garbowski (**3**) who found it associated with roots of alfalfa infected by *Sclerotinia trifoliorum* Eriks. It has not been reported previously from the United States.

The dark-setose species differs from these four in the color of the setae and the spore size as well as in the structure of the sporodochium. It is apparently new.[3]

[1] Journal Paper No. J-2442 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1018.

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[3] The author wishes to express gratitude to Edith M. Cash for help with the Latin description.

Volutella colletotrichoides sp. nov.

Sporodochiis sessilibus, primitus hemisphaericis, demum erectis, cylindratis, 40–70 μ diam., externe 40–110 μ, extendientibus, rubentibus ad carneis, setis marginalibus erectis paucis praeditis. Setis nigris, 2–4-septatis, 60–110 × 4–6 μ. Sporophoris continuis, simplicibus, 18–40 × 2–3 μ, in stratum densum e basi mycelii laxe aggregati parallele dispositis. Conidiis hyalinis, continuis, navicularibus, 5.2–10 × 2–3.5 μ.

Habitat: In ramulis *Medicaginis sativae, M. falcatae, Trifolii pratensis, T. hybridi;* Iowa, U.S.A.

Sporodochia sessile, at first hemispherical, then erect cylindrical, 40–70 μ diameter, extending outward 40–110 μ, pink to flesh-colored, with few marginal erect setae. Setae black, 2–4-septate, 60–110 × 4–6 μ, tapered. Conidiophores simple, 18–40 × 2–3 μ, forming a dense parallel layer on a base of loosely aggregated mycelium. Conidia hyaline, single-celled, navicular, 5.2–10 × 2–3.5 μ.

(On Czapek's agar forming a pink to brownish appressed mat, with numerous sporodochia developing. Partially deficient for biotin.)

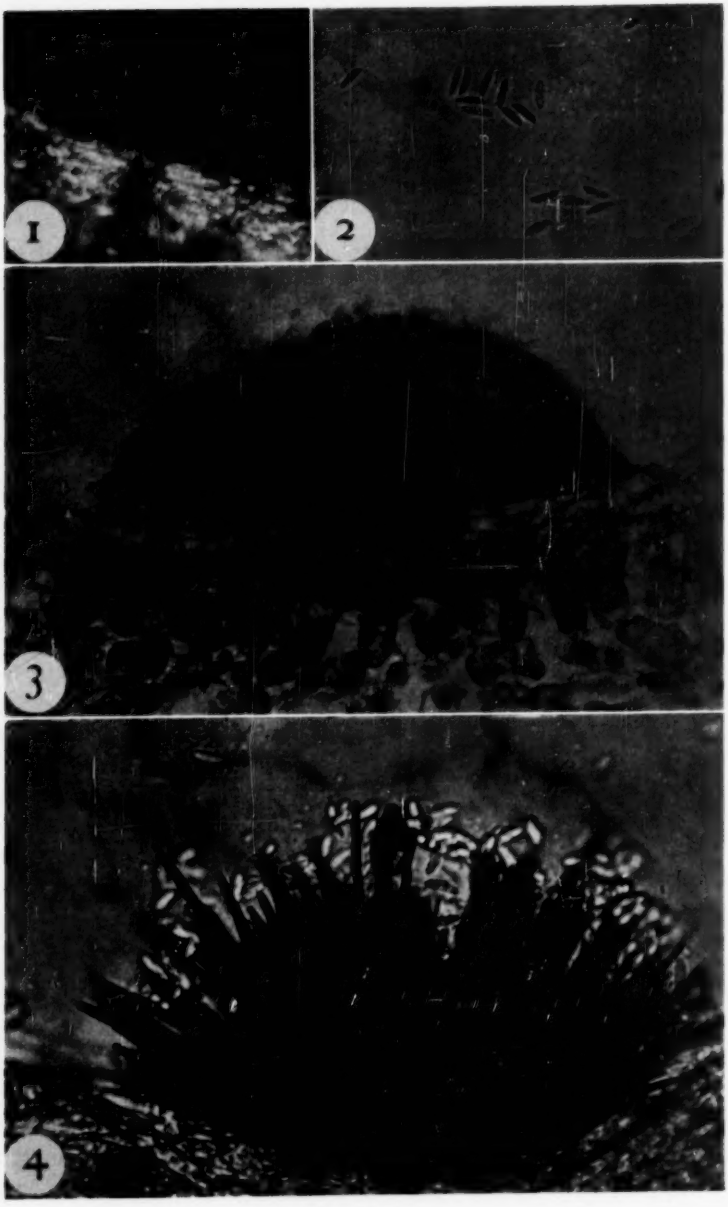
VOLUTELLA COLLETOTRICHOIDES var. **setosa** var. nov.

A *V. colletotrichoides* differt setis numerosioribus.

Identical in conidial size and shape with typical form; sporodochia densely setose.

The fungus newly described herein shows interesting relationships with both the genera *Colletotrichum* and *Volutella*. Sporodochia and acervuli, the historic criteria of the two genera, are not always clearly separable. Some investigators (**2**, **6**, **8**) have placed marginal species in *Colletotrichum* on the basis of dark setae, innate origin of conidiophores, and large conidia, reserving *Volutella* for pale or hyaline colored, superficial sporodochia with small conidia.

V. gilva and *V. ciliata* present two distinct types of sporodochial development which agree with the above interpretation of *Volutella*. The former initiates a layer of conidiophores and hyaline setae from a superficial mycelial network; the latter forms conidiophores on a pseudoparenchymatous stipe of innate origin. The development of *V. colletotrichoides* is more obscure. The cushion-shaped mycelial base varies from very small to large and stipe-like, and protrudes above the surface of the tissues. The conidiophores and setae which arise from this base were not observed to be innate in origin. The small conidia, which are well within the range of *Volutella* species and smaller than those of *Colletotrichum*, along with the apparent superficial nature of the fruiting structures, appear to relate the fungus more closely to the genus *Volutella*.



FIGS. 1–4.

Sporodochial development was observed by the use of freehand sections of naturally infected tissues and microtome sections of inoculated alfalfa petioles and leaflets. The latter were fixed in Craf III solution at various stages of development, and dehydrated and infiltrated with dioxan-normal butyl alcohol. Iron hematoxylin and fast green provided adequate staining.

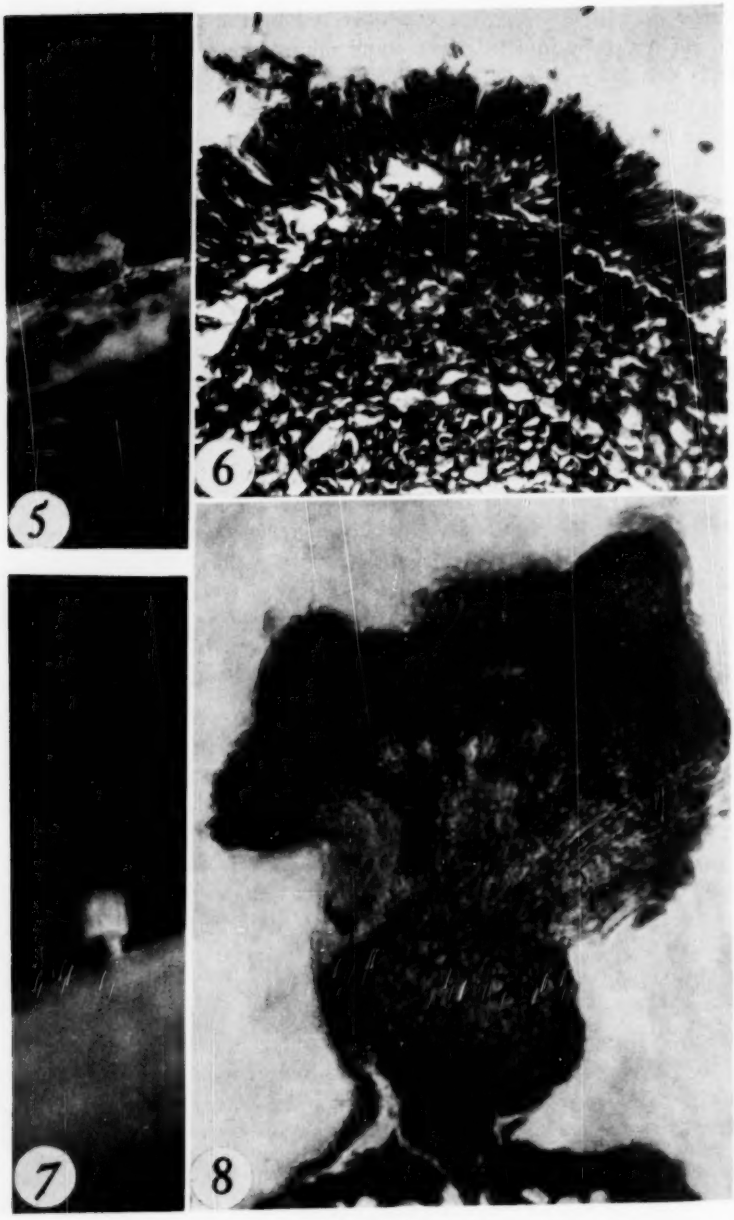
The fruiting bodies first appeared as hemispherical, flesh to pink colored masses which often developed into erect, cylindrical, sometimes recurved sporodochia, measuring 40–70 μ in diameter and extending to a height of 40–100 μ (FIG. 1). The setae, usually numbering 5–25 per sporodochium, were dark-brown, 60–110 × 4–6 μ, 2–3-septate, tapered, primarily marginal. The conidia, produced in a mucus-imbedded mass, were hyaline, single-celled, navicular, measuring 5.2–10.5 μ in length (FIG. 2).

Sparse mycelial development occurred in the epidermis and internal tissues. The sporodochium was initiated by the extrusion of a mycelial tuft through the epidermis, from which a dense layer of parallel, simple, non-septate conidiophores 18–40 × 2–3 μ developed, radiating outward to give a distinct fan-shaped appearance in median section (FIG. 3). The mycelial base protruding through the epidermis was so distinct as to suggest a minute stipe. The setae originated at or near the origin of the conidiophores.

Conidia germinated in 6–8 hours on artificial media, producing an appressed yellow or pale pink colony in 36 hours and aften producing conidia from short branches in 30 hours. As setose sporodochia developed, the colony became flesh colored. On biotin-fortified Czapek's agar the colony became an intense brownish-red with black pigmented zones.

An isolate obtained from field-inoculated alfalfa plants differed from the above described fungus by the production of a large number of setae per sporodochium (FIG. 4). The conidia were similar in shape and size. On PDA growth was similar to the original strain except for production of more setae. On Czapek's agar the growth of this more densely setose strain was roughened in contrast with the smooth growth of the original form. Both strains reacted similarly in reaction to various temperatures and both strains required exogenous biotin for growth. This densely setose isolate remained distinct through numerous cultural treatments and was considered as warranting varietal rank.

FIGS. 1–4. *Volutella colletotrichoides*. 1. Sporodochium, habit, × 18. 2. Conidia, × 450. 3. Transverse section of sporodochium, × 385. 4. Transverse section of sporodochium of variety *setosa*, × 450.



FIGS. 6–8.

VOLUTELLA GILVA (Pers. ex Fr.) Sacc.
Conoplea gilva Pers.
Psilonia gilva (Pers.) Fr.

On naturally infested plant tissues sporodochia of *V. gilva* first appeared as white tufts of mycelium. As the fruiting body developed the structure became strongly pulvinate, often with perpendicular sides, circular, oblong or irregular in outline (FIG. 5). The caespitose sporodochia were 1–3 mm broad, extending outward up to 1 mm. Conidia, produced in a mucous mass, gave a bright, rosy color to the structure. The conidia were cylindric, with slightly curved sides and obtuse ends, 9–12 × 2–3 μ with a mean length of 9.5 μ. The setae, both marginal and scattered on the sporodochium, were hyaline, flexuous, multiseptate, tapered, 140–210 × 5–7 μ.

Mycelium readily penetrated throughout the tissues of alfalfa petioles. The sporodochium was initiated by the emergence of hyphae to the surface where they branched to form a loose, surface network of mycelium. Unicellular simple conidiophores formed a dense sporogenous surface over this network, producing the large spore mass (FIG. 6).

Inoculation on PDA resulted in pure white fluffy mycelium forming a rapidly growing colony. Conidia often developed sparsely over the surface, giving a pinkish tinge to the colony. Sporodochia appeared within the colony as erumpent rosy red masses.

VOLUTELLA CILIATA (Alb. & Schw.) Fr.
Tuberculariа ciliata Alb. & Schw.
Psilonia rosea Berk.
Artractium ciliatum Link.
Fusarium ciliatum Link.

V. ciliata first appeared on plant tissues as small white mycelial clumps, gaining a slimy appearance from massing of conidia. The sporodochia enlarged rapidly, often becoming stipitate, the conidia forming a hemispherical circular cap on the stipe (FIG. 7). Individual sporodochia measured 200–400 μ long, with marginal hyaline, tapered, rigid setae, 325–375 × 4–5 μ. The conidia were hyaline, unicellular, elliptical, with obtuse ends, 4–9 × 1.5–2.5 μ, the majority measuring 6 μ. Usually a central single globule was discernible in each conidium.

FIGS. 5–8. 5. *Volutella gilva*, sporodochium, habit, × 16. 6. Transverse section of sporodochium, × 385. 7. *Volutella ciliata*, sporodochium, habit, × 32. 8. Transverse section of sporodochium, × 450.

Mycelium development in the cortex of alfalfa petioles obliterated the parenchyma tissues. A parenchyma-like fungus stroma developed within the tissues, erupting to the surface to form the stipe of the sporodochium. Unbranched, two- to three-septate conidiophores developed over the surface of this basal stipe, forming a dense layer of parallel strands, 35–45 × 2–3 μ (FIG. 8). The production of conidia often became so massed as to obscure the stipe.

Inoculation on PDA resulted in a closely appressed growth, forming a thin gray mycelial pad over the agar surface, usually showing a zonate pattern. Conidia appeared in distinct sporodochia arising from the mat, imparting a distinct yellow cast. Coalescing of sporodochia formed large clumps of spores.

PATHOLOGY

The pathological nature of *Volutella colletotrichoides* was determined by observations of naturally infested greenhouse and field plantings and by infection experiments in the field and greenhouse. *V. gilva* and *V. ciliata* were tested for pathogenicity in a number of experiments with negative results.

The original isolations of *V. colletotrichoides* were made from greenhouse-grown forage legume accessions of the Plant Introduction Station which had been transferred as stem cuttings from the 1949 field plantings. Four samplings of these were incubated in moist chambers and microscopic determination of infestation made. Of 36 accessions thus sampled, eleven percent yielded *V. colletotrichoides*, the fungus commonly being associated with petiole lesions. Common alfalfa yielded the most isolates, but red clover and birdsfoot trefoil also produced the fungus.

During the summer of 1950 and 1951 periodic observations and samplings of field grown Plant Introduction accessions were made. The results of these observations showed a sparse and erratic occurrence, with little change in the incidence during any part of the season or during the two years. Two years sampling of several alfalfa and red clover fields in the vicinity were negative, showing the confinement of the fungus to the Plant Introduction plots.

Conidial inoculations were made in the spring of 1950 on small field plots of alfalfa and red clover. A small but consistent rate of recovery of the fungus from infected plants occurred during 1950 and 1951, indicating the ability of the fungus to maintain itself successfully.

Observations were made on Plant Introduction stocks at the Nebraska Agricultural Experiment Station in September, 1951. Three groups of

plants sampled were: (1) greenhouse-grown accessions, obtained as stem cuttings from the 1949 plantings at Ames; (2) field-grown accessions from seed of the same lots as the 1949 plantings at Ames; and (3) field-grown accessions from 1950 Plant Introduction seed lots. Fragmentary plant parts of the greenhouse plants available failed to yield the fungus. The seven accessions of the 1949 field plantings sampled (which were known to be infested at Ames) yielded the fungus in all cases. None of the 1950 plantings (located one mile from the 1949 plots) yielded the fungus.

Greenhouse inoculations were carried out primarily on alfalfa and red clover. The plants were grown in 4 or 8 inch pots in a sand-peat-soil mix. Autoclaved soil was used for certain soil inoculation tests. Supplementary illumination was added during winter months. Soil inoculum was prepared by mixing either PDA or oat cultures with soil. Seed was inoculated by immersing previously disinfected seed in conidial suspensions and drying between sterile filter paper sheets. Foliage inoculations were made by spraying conidial suspensions with a De Vilbiss hand atomizer. Normally plants were pretreated by covering with fog spray 2 hours prior to inoculation. Moist chamber incubation varied from 24 to 48 hours.

Three replicated experiments involving soil and seed inoculation produced no root infections. Girdling of young alfalfa seedlings, resulting from linear, water-soaked stem lesions, did result from such inoculations to a very limited extent. Laboratory studies, in which conidial suspensions were added to sterilized sand in deep Petri plates prior to planting disinfected red clover seed, showed similar stem infections. *V. colletotrichoides* was recovered from such infections.

Spray inoculations of seedlings resulted in a small but consistent rate of infection. Common symptoms on both alfalfa and red clover were water-soaking at some point on the petiole with elongate lesions and girdling resulting. Symptoms usually occurred 5 to 10 days after inoculation.

Inoculations on mature alfalfa and red clover plants produced petiole girdling and girdling of the stem tips, which caused a blighted appearance of severely infected plants. Infection of mature stem tissues produced elliptical sunken lesions to a limited extent. No evidence of systemic invasion of the plant was found although stem infections gradually enlarged to involve much of the plant under high moisture conditions. Isolations were made from necrotic and spotted leaflets but leaf spotting was not a consistent or definable symptom.

Successful isolations of *V. colletotrichoides* were made from *Medicago*

sativa, Trifolium pratense, T. hybridum and *Lotus corniculatus* from the field. Greenhouse inoculations established infection on the first three as well as on *Medicago falcata*. *Melilotus alba* and *Glycine max* were not infected by the fungus. Common alfalfa appeared to be the most susceptible.

All infection studies showed a very low incidence of infection. Such factors as age of inoculum and condition and age of host plants made little change in this low infectivity. Varietal susceptibility of alfalfa and red clover was determined in greenhouse inoculation experiments. All varieties tested were susceptible, but no extreme susceptibility appeared in any variety.

Plant pathogens introduced into a new population have been among the most serious causes of disease. The evidence obtained indicates that *V. colletotrichoides* was introduced to the area on forage legume seed planted by the Plant Introduction Station in 1949. The fact that it was confined to Plant Introduction stocks at Ames, and Lincoln, Nebraska, and has not previously been reported on a pathologically well known crop such as alfalfa tends to confirm this. Conidia were found to be viable for over one year on stored alfalfa seed, and infection from seed inoculation was obtained in experimental work.

The lack of any noticeable spread or increase during two years, plus the light infection obtained on a number of varieties of both alfalfa and red clover, indicates little likelihood of the fungus menacing forage production.

SUMMARY

Three species of *Volutella* infest forage legumes in the Ames vicinity. Two of these, *V. gilva* and *V. ciliata*, are saprophytes, the latter not previously reported from the U.S. The sporodochia are bright colored and superficial, typical of the genus. The third species, *V. colletotrichoides*, a newly described facultative parasite of several forage legumes, is believed to have been introduced with seed lots of the Plant Introduction Station. This fungus, which produces sparse black setae and superficial sporodochia, was distinguished from the genus *Colletotrichum* because of the small conidia and superficial nature of the fruiting body. Several isolates from field-inoculated alfalfa consistently produced densely setose sporodochia but did not differ in conidial size and shape. This form was named *V. colletotrichoides* var. *setosa*.

V. colletotrichoides did not show indication of severe pathogenicity to any plant tested. No detectable spread or increase in the field dur-

ing two years was observed, indicating little likelihood of the fungus becoming of economic importance.

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AN UNDESCRIBED SPECIES OF PIRICULARIA ON SEDGES [1]

E. S. Luttrell

(WITH 3 FIGURES)

In June 1950 Dr. B. B. Higgins found in Griffin, Georgia, specimens of nutgrass (*Cyperus rotundus* L.) which were heavily infected with a previously unrecorded leaf spot. Subsequently, this disease was found, although less abundantly, on nutgrass in fields in the vicinity of Griffin. In October 1951 a similar leaf spot on *Cyperus* sp. was found in cultivated fields near Tifton, Georgia. Study of the fungus associated with the spots indicated that it is an undescribed species of *Piricularia*. The fungus is named for Dr. Higgins, who first collected it, and is described as follows:

Piricularia higginsii sp. nov.

Maculis foliorum ellipticis, brunneolis, fusco-marginatis, amphigenis, subinde confluentibus, 1.5–7 × 0.5–2 mm; conidiophoris copiosis, solitariis vel gregariis, per epidermidem erumpentibus, erectis, simplicibus, sursum attenuatis, continuis vel 3-septatis, hyalinis vel fuligeneis, apice 2–4-denticulato, proliferis, 30.4–76.0 μ longis, basi 3.0–6.8 μ diam., apice 1.8–2.7 μ diam.; conidiis acrogenis, stipitellatis, e denticulis conidiophoris oriundis, hyalinis, obclavatis, basi rotundatis apiculatis, ad apicem obtuse attenuatis, rectis, levibus, 2-septatis, non constrictis, 17.5–36.5 × 5.3–6.5 μ (media 26.1–28.6 × 6.0–6.1 μ).

Hab. in foliis vivis *Cyperi rotundi* L. et *Cyperi* sp., Georgia, U. S. A.

Collections: On *Cyperus rotundus*, Griffin, Georgia, 15 July, 1950, E. S. Luttrell. Type: Bureau of Plant Industry Mycological collections, Beltsville, Maryland. Co-types: Farlow Herbarium, Harvard University; Herbarium of the Royal Botanic Gardens, Kew; Herbarium of the Institute of Systematic Botany, Uppsala. On *Cyperus* sp., Tifton, Georgia, 2 October, 1951, E. S. Luttrell. Bureau of Plant Industry Mycological collections.

[1] Paper No. 254, Journal Series, Georgia Experiment Station.

I am indebted to Mr. John A. Stevenson and Dr. S. J. Hughes for advice on the classification of the fungus and to Dr. Donald P. Rogers and Dr. Richard P. Korf for permitting me to examine collections in the Herbarium of the New York Botanical Garden and the Herbarium of the Department of Plant Pathology of Cornell University.

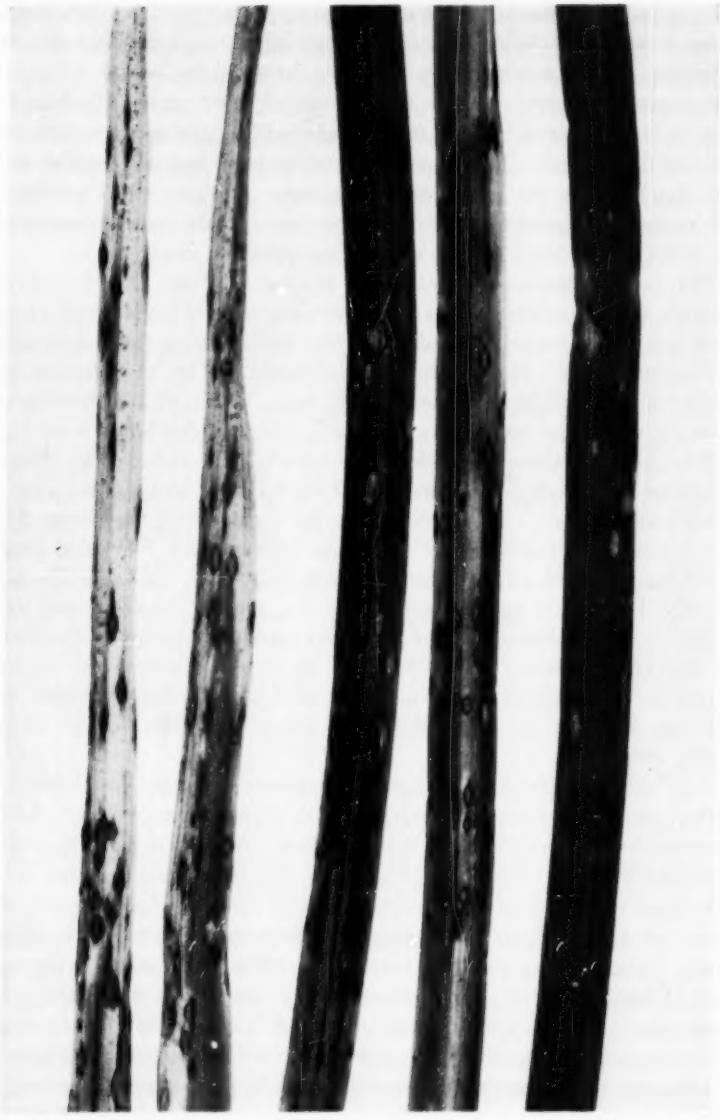


FIG. 1. Leaves of *Cyperus rotundus* infected with leaf spot caused by *Piricularia higginsii*.

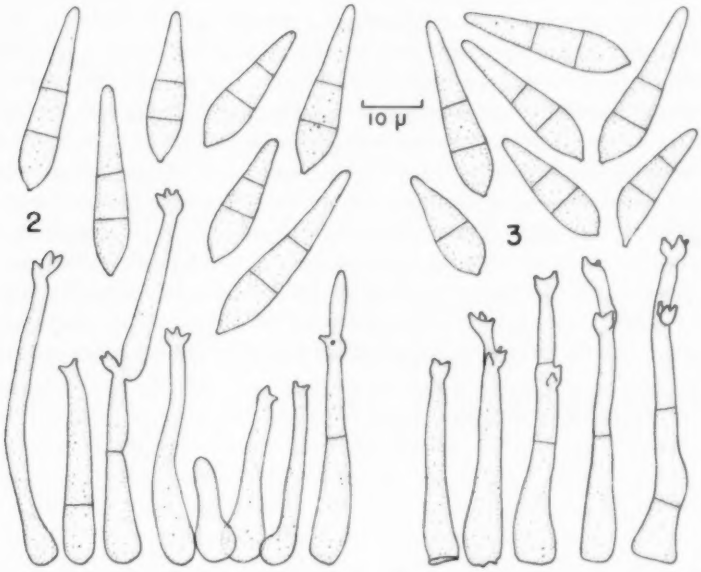
On *Cyperus rotundus* the lesions first appeared as tiny, dark brown flecks in the leaf blade. The lesions enlarged to form elliptical, pale brown spots surrounded by a narrow, dark brown border (FIG. 1). The spots measured 1.5–7 × 0.5–2 mm. Under moist conditions a gray, velvety layer of conidiophores and conidia developed on both surfaces of the lesions. The spots coalesced to form larger irregular spots and blotches, and the leaves died back from the tips. On *Cyperus* sp. the spots were smaller and more nearly circular, hardly exceeding 1.5 × 0.8 mm. Most of the leaves were partially dead.

The conidiophores of *Piricularia higginsii* (FIGS. 2, 3) emerged through the epidermis. They occurred singly or in small clusters and were simple, somewhat inflated at the base and tapering toward the apex, 0–1-septate or, in older proliferous conidiophores, up to 3-septate, and hyaline to pale fuliginous. At the apex was a crown of 2–4 short sterigmata on which the conidia were borne. The conidiophores were often proliferous, producing 2–3 successive whorls of conidia. On *Cyperus rotundus* the conidiophores were 30.4–63.8 μ long and 4.6–6.1 μ in diameter at the base. In the collection on *Cyperus* sp. they were 35.0–76.0 μ long, and the diameter varied as follows: base 5.6–6.8 μ, middle 3.0–4.3 μ, apex 1.8–2.7 μ. The conidia were hyaline, obclavate, 2-septate (rarely 1-septate), not constricted at the septum, smooth, and thin-walled. A protuberance at the basal end marked the point of attachment to the conidiophore. Measurements were as follows: On *Cyperus rotundus* 24.3–36.5 × 5.3–6.5 μ, mean 28.6 × 6.0 μ, length/width ratio 4.8; on *Cyperus* sp. 17.5–33.4 × 5.8–6.4 μ, mean 26.1–6.1 μ, length/width ratio 4.3.

Piricularia higginsii was obtained in culture by single-conidium isolations from the material on nutgrass. On 2 percent malt agar it formed cream-colored, wrinkled, yeast-like colonies which at first sporulated abundantly. After several transfers, the fungus formed a sparse, white, flocculent mycelium and ceased to produce conidia. Inoculations were made on vigorously-growing nutgrass plants completely filling six-inch pots. Inoculum consisted of malt agar cultures macerated with water in a Waring Blendor. The inoculum was sprayed on the plants, which were then placed in a moist chamber for 48 hours. Plants in three pots were inoculated, and the same number served as uninoculated controls. At the end of one week all inoculated plants were heavily infected, exhibiting typical leaf spot symptoms. The leaves photographed for FIG. 1 were taken from these plants. There was no infection on any of the control plants. No inoculations were attempted on *Cyperus* sp.

The sedge fungus differs from *Piricularia grisea* (Cke.) Sacc., the

type species of the genus *Piricularia*, in that the conidia occur in whorls of 2–3, or rarely 4, at the apex of the conidiophore. However, the conidia in *P. grisea* are often so closely grouped that they appear to be produced in heads. The sedge fungus differs also in that the conidia are abstricted from short sterigmata. In *P. grisea* the conidia are likewise borne on short stipes, but these stipes resemble disjunctor cells. The stipe cell ruptures in the middle, leaving a protuberant collar on the conidiophore and a similar collar at the base of the conidium. The



FIGS. 2, 3. *Piricularia higginsii*—conidia and conidiophores. 2. From *Cyperus rotundus*. 3. From *Cyperus* sp.

conidia of the sedge fungus might be considered capitate, and this character suggests the genus *Dactylaria*. It should be noted that *P. grisea* and many of the related species on grasses have at one time or another been placed in *Dactylaria*. It seems that the obpyriform to obclavate shape of the conidia should be the primary character in separating *Piricularia* from related genera such as *Dactylaria*, *Dactylella*, and *Ramularia*. Because of the obclavate conidia and parasitism on a vascular plant, the sedge fungus is placed in *Piricularia*.

Piricularia higginsii differs from all other described species of *Piricularia* in its longer, narrower conidia. It is the only species in the genus

with a conidial length/width ratio in excess of 4. The maximum diameter of the conidia is less than 7 μ, whereas in *P. grisea* and the numerous other closely related, and possibly synonymous, species on grasses the minimum diameter is 7 μ. *P. scripta* (Bon.) Sacc. differs in its 4–5-septate conidia and occurrence on tree branches and fruit. *P. parasitica* Ell. & Ev. is the only species which approaches *P. higginsii* in conidial dimensions. Examination of the type collection of *P. parasitica* showed that the conidia are short-cylindric to fusoid rather than obclavate and the conidiophores are very long and branched. Furthermore, it appears to be a hyperparasite on *Phyllachora graminis* (Pers. ex Fr.) Fckl. *P. parasitica* probably should not be retained in *Piricularia*. It might be placed in *Ramularia*. In *P. euphorbiae* (Tracy & Earle) Atk. the conidia are ovoid to clavate-ovoid, and the long, brown conidiophores arise in large clusters from massive stromata. This species has been classified in *Cercosporidium* (= *C. fasciculatum* (Cke. & Ell.) Hoehn.) and might ultimately be placed in the genus *Didymaria* (Corda) emend. Solheim. *P. caudata* Appel & Str. is distinguished by its fusoid conidia which bear an apical seta and has on this account been transferred to *Trichoconis* as *T. caudata* (Appel & Str.) Clem. *P. aquatica* Ingold and *P. submersa* Ingold differ strikingly in habit and in their fusoid conidia. These two species might more properly be placed in *Dactylella*.

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THE GENUS ARTHRINIUM [1, 2]

WM. BRIDGE COOKE [3]

Investigation of the status of species of *Arthrinium* found in the Pacific Northwest disclosed several taxonomic problems in this and related genera. As it was deemed advisable to observe more material before attempting the solution of these problems, specimens were received on loan from the New York Botanical Garden through D. P. Rogers and from the Mycological Collections, Bureau of Plant Industry, through J. A. Stevenson. In addition, the writer's collections, those housed in the Plant Pathology Herbarium at the State College of Washington and those at the Herbarium of the University of California, were studied. D. P. Rogers, W. W. Diehl, C. G. Shaw, and Lee Bonar have assisted in the clarification of ideas as different phases of the problem developed.

The genera *Goniosporium*, *Gonatobotryum*, and *Arthrinium*, assigned by Lindau (in Rabenhorst's Kryptogamen-Flora **1** (8), 1907) to his tribe *Arthrinieae*, recently have been rearranged by E. W. Mason (Annotated account of fungi received at the Imperial Mycological Institute, List II, fasc. **2**: 26–29. 1933). Mason was especially interested in the genus *Papularia* Fr. and its close relatives. He placed together all dematiaceous fungi with flattened spores which were of various shapes, dark in color, and one-celled. His new tribe, *Bivalvae*, was based on *Papularia*, the spores of which have a Saturn-like ring at which they appear to open (like a clam shell) on germination or on crushing. In the tribe *Bivalvae* he included four other genera: *Arthrinium*, *Camptoum*, *Goniosporium* and *Pseudocamptoum*. In the present discussion neither *Papularia* nor *Pseudocamptoum* Frag. & Cif. is considered in detail because of their obvious dissimilarities with the other three genera. Lindau included *Camptoum* in his tribe *Periconieae*, presumably because the spores were clustered at the top of the conidiophore—an

[1] This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

[2] Scientific Paper No. 1055, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, the State College of Washington, Pullman.

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arrangement due to the preparation of poor water mounts rather than to the true morphology of the organism. Lindau had also included *Gonatobotryum* Sacc. in the *Arthrinieae*. Mason disagreed with this, since *Gonatobotryum* is quite different, and on this basis he considered the group artificial and worthy of breaking up and renaming. *Arthrinium* and *Goniosporium* were separated by Lindau and by Mason on quite arbitrary and, to this writer, artificial bases. *Camptoum* was also maintained separately on artificial bases. From the examination of conidiophores, conidia, and the method of conidial production, it seems to the writer that the retention of these three genera as distinct is not correct. The genus *Arthrinium* is suggested as the genus in which all should be included. This is the only one of these genera used by Fries, who followed Link's conception of the group. *Arthrinium*, rather than being placed in the tribe *Bivalvae* with *Papularia*, should remain in the long established *Arthrinieae* as Lindau had it, according to a strict interpretation of the International Code of Botanical Nomenclature.

Another solution to the problem of the position of *Arthrinium* in the classification of the Hyphomycetes has been offered recently by Hughes (Can. Jour. Bot. **31**: 577–659. 1953). Hughes divides the Hyphomycetes into eight sections regardless of Saccardoan family lines, which are eliminated. These eight sections are divided into groups on the basis of: "growth of conidiophore initial or of conidiophore during sporulation restricted to apical region," and "growth of conidiophore restricted to basal region." The only section included in the second of these categories is Section VIII. The few genera mentioned include *Papularia, Arthrinium, Endocalyx, Dictyoarthrinium, Spegazzinia,* and *Graphiola*. The section description follows: "In this section are included those species whose conidiophores elongate by a basal growing point. The conidiophore itself may arise from a barrel-shaped or flask-shaped conidiophore mother-cell which usually bears a terminal, often atypical conidium prior to the development of the conidiophore. The conidia of mature conidiophores are either borne solitarily at the apex or apically and irregularly on short stalks along the increasing length of the conidiophore, or apically and in whorls between thickened septa of the conidiophore. All the conidia arise as blown-out ends and the oldest conidia are usually towards the apex and the youngest towards the elongating base of the conidiophore." Hughes considers this type of conidiophore a "basauxic conidiophore."

In examining nearly 50 collections of specimens assignable to species of *Arthrinium*, it was found in all cases that at maturity the conidia, rather than being terminal, are seated along the conidiophores; and

that rather than being produced on phialides or phialid-like structures, are essentially sessile; rather than being seated at one end or the other of the spore, the spores are seated in the middle; rather than being produced on the dark cross-walls in the conidiophore they are produced on the hyaline cells between the dark cross-walls. In a personal communication, W. W. Diehl states that in at least one specimen the spores appear to be produced at the tip of the conidiophore. As the spore matures the point of production appears to become lateral, giving way to the production of another spore at the tip. To the writer this observation appeared to be confirmed in other specimens. In mounts prepared with phloxine and KOH it can be seen easily that in the hyaline cell the wall is rather thick, the lumen is filled with red staining material even after the spore is released or removed by mechanical injury in mounting, and the lumen is continuous to the point at which the spore is produced, thus indicating that a pore is formed in the conidiophore cell wall at the point of spore formation. It is suggested that other interpretations resulting in the recognition of at least three genera in this group of species are based on misinterpretations produced by mounting techniques which may not have permitted adequate observation of conidia and conidiophores.

Apparently the spore production mechanism in this genus and tribe represents an advance of the phialid from a simple, one-celled structure, to a complex multi-celled structure. Ellis *et al* (Trans. Brit. Myc. Soc. **34**: 497–514. 1951) indicate that at the base of the hyphal branch on which the spores are produced there is a phialid-like cell in both *Arthrinium* and *Papularia*. This cell lies on one of the complex of hyphae in the undifferentiated mycelium.

If one restricts the term phialid, as does Hughes, to types of spore-producing structures belonging to genera assigned to his Section IV, then *Arthrinium* has no phialides. An interpretation based on Hughes's work of the conidia and conidiophores described above follows. This interpretation makes certain factors more understandable. While older herbarium specimens do not show all the features observed in relatively fresh, recent collections, extrapolation can be used for interpretation of the structures. The atypical spore at the tip of the conidiophore on the conidiophore mother cell can be found in all species described below of which adequate material was available for study. This spore, although differing from the typical spores of the species, is similar between the several species studied. The more mature spores toward the tip of the conidiophore are uniformly opaque while the less mature spores toward the middle and lower part of the conidiophore are less opaque and in

the younger spores the cytoplasm in the spore and in the bridge between the spore and the conidiophore is visibly stained with phloxine when mounts are made with that stain.

L. S. Gutner, cited by Ellis *et al* (l.c.), was the first to produce mature perithecia, asci, and ascospores in culture using conidia of *Camptoum curvatum* Lk. This fungus was found on *Scirpus* but was also grown in culture on autoclaved leaves from eleven monocot and one dicot hosts. The perfect state was named *Pseudoguignardia scirpi* by Gutner. Culture work was repeated by Ellis and his co-workers using British material of this species with similar results. Their new variety *minus* was characterized by smaller ascospores in nature. This variety produced only a perfect state in pure culture, which was also characterized by smaller spores.

It should be noted that one source of confusion in this group is the lack of original illustrations. The following may be only a coincidence, but it is suggestive of the situation throughout the imperfect fungi. *Camptoum curvatum* was illustrated by Corda (Icon. **3**: *7, f. 17.* 1839) and this illustration was picked up by Lindau (l.c. p. 633). In 1933 Mason illustrated this fungus on page 28 with a figure credited to Ferraris which is almost an exact copy of the Corda figure reproduced by Lindau. Recently Ellis *et al* (l.c.) have illustrated the commoner species of this genus with excellent line drawings.

ARTHRINIEAE Lindau, Rabenhorst Krypt.-Fl. I. **8**: 635. 1907.
Bivalvae Mason, Imp. Myc. Inst. List II, fasc. **2**: 29. 1933.

Spores dark in color, flattened, seated on the sides or tips of short to elongated conidiophores, conidiophores simple hyphal branches subtended by a phialid-like cell, decumbent or erect, with hyaline cells separated by dark cross-walls.

KEY TO GENERA

1. Spores round in face-view outline, with a Saturn-like ring, produced on simple hyphal branches ..... *Papularia*
1. Spores of various shapes, without a Saturn-like ring, produced on hyaline cells of erect conidiophores ..... *Arthrinium*

ARTHRINIUM Kze. ex Kze. ex Fr., Syst. Myc. **3**: 375. 1832.
Arthrinium Kze. ex Kze., Myk. Hefte **2**: 101. 1823.
(First published in Myk. Hefte **1**: 9. 1817.)
TYPE: *A. caricicola* Kze. ex Fr., l.c.

Camptoum Lk. in Willdenow, Linn., Sp. Pl. 4 ed. **6**(1) : 44. 1824.
TYPE: *C. curvatum* Lk., l.c.
Goniosporium Lk. in Willdenow, Linn., Sp. Pl. 4 ed. **6**(1) : 44. 1824.
TYPE: *G. puccinioides* Lk., l.c. p. 45.
Sporophleum Lk. in Willdenow, Linn., Sp. Pl. 4 ed. **6**(1) : 45. 1824.
TYPE: *S. gramineum* Nees ex Lk., l.c. p. 45.
Gonatosporium Corda, Anleit. 42. 1842.
(The writer prefers to consider this a mis-spelling of *Goniosporium* Lk. since Corda, l.c., refers to "*Gonatosporium* Lk. p. 44.")
TYPE: *Goniosporium puccinioides* Lk., l.c.
Turcenia Hall, Phytopathology **5**: 57. 1915.
TYPE: *T. juncoidea* Hall, l.c.

Perfect state: (proved for one species only)
Pseudoguignardia Gutner, Mat. Myk. Fitop. **6**: 311. 1927. (Cited in Clements and Shear, 1931, as a synonym of *Phomatospora* Sacc., Fungi Ven. **2**: 306. 1874.)
TYPE: *P. scirpi* Gutner, l.c.

Forming black pustules or indeterminate areas of conidial production on dead leaves and culms of various herbaceous plants. Pustules or spore-producing areas formed of masses of conidiophores with few basal hyphae. Conidiophores erect, black, formed of usually isodiametric cells from each of which one to four spores are produced, cells hyaline, thick-walled, with a pore at the point of origin of each spore, separated by dark brown to black septa which appear as thickened bands; spores sessile, seated in the center, attached laterally on the conidiophore cells, variously shaped, dark brown, dark olive green, or black, flattened, where tips are present these may be hyaline.

1. ARTHRINIUM CARICICOLA Kze. ex Kze. ex Fr., Syst. Myc. **3**: 375. 1832.
Arthrinium caricicola Kze., Myk. Hefte **1**: 9. 1817. (Combined genus and species description.)
Arthrinium caricicola Kze., Myk. Hefte **2**: 102. 1823.
Arthrinium naviculare Rostrup, Bot. Tidsskr. **15**: 235. 1886.

Spores at first produced at the tip of the conidiophore, appearing like those of *A. cuspidatum*, then becoming flattened and drawn out into the shape of an elongated diatom and seated on the side of the conidiophore cell, ultimately cigar-shaped or diatom-shaped, brown, (36)–42–47–(54) × 9–12 μ.

Habitat: On dead leaves of *Carex* spp.
Distribution: Europe: Austria, Germany, Finland, Russia.

2. ARTHRINIUM CUSPIDATUM (Cke. & Harkn.) von Hoehnel, Mitt. Bot. Inst. Techn. Hochsch. Wien **2**: 9–16. 1925.
Camptoum cuspidatum Cke. & Harkn., Grev. **12**: 33. 1883.
Arthrinium bicorne Rostrup, Bot. Tidsskr. **15**: 235. 1886.
Tureenia juncoidea Hall, Phytopathology **5**: 57. 1915.

Spores averaging 21.6 μ long, with horn-like tips 7 μ long, the body of the spore 10 μ wide.

Habitat: On dead leaves and culms of *Juncus* and *Carex* species.

Types examined: *Camptoum cuspidatum* Cke. & Harkn.; *Tureenia juncoidea* Hall.

Distribution: North America: California: Mount Shasta, Yosemite National Park, and San Francisco; Colorado: Mammoth; Washington: Pullman; Wyoming: Medicine Bow Mountains; Europe: Sweden, Russia.

In a cultural study of one of the Mount Shasta collections, Bonar found that in a windowbox in Berkeley a pure culture fruited abundantly in daylight; a subculture kept in the dark produced mycelium but no spores. See description by W. B. Cooke, Mycologia **41**: 603. 1949.

In 1915 John G. Hall described (Phytopathology **5**: 57. 1915) a species which he named *Tureenia juncoidea* on dead rush stems collected at Pullman, Washington. He reported that parts of the type were deposited at the Farlow Herbarium, the New York Botanical Garden, and the Mycological Collections of the Bureau of Plant Industry. Only the portion housed in the latter herbarium was studied by the present writer. The spores of this species, which were well illustrated in the cited article, proved to be identical with those of the species commonly referred to in this country as *Arthrinium bicorne* Rostrup, recently illustrated by the writer in Mycologia **41**: 603. 1949.

Much earlier M. C. Cooke and Harkness (Grev. **13**: 33. 1883) had described a specimen collected by Harkness in California on the leaves of *Scirpus* as *Camptoum cuspidatum*. This material was collected in a cemetery, now a residential section, in San Francisco. Von Hoehnel later referred this species to *Arthrinium*. The type was kindly loaned by John Thomas Howell from the Harkness collection at the California Academy of Sciences, San Francisco.

Examination of the types of *Tureenia juncoidea* and *Camptoum cuspidatum* and of several specimens identified as *Arthrinium bicorne* Rostrup

(published in 1886; unfortunately Rostrup's type was not available) indicates that the specimens are cospecific. Since the name applied by Cooke and Harkness has priority the name of the species becomes *Arthrinium cuspidatum* (Cke. & Harkn.) von Hoehnel.

3. ARTHRINIUM SPOROPHLAEUM Kze. ex Fr., Syst. Myc. **3**: 377. 1832.
Sporophleum gramineum Kze., Myk. Hefte **2**: 105. 1823.
Arthrinium sporophlaeum Kze., Myk. Hefte **2**: 104. 1823.

A well-marked species, so far as can be determined from material available. Specimens of other species have often been mistaken for it, and only one collection was labeled correctly among those examined. Spores brown, broadly ovate to lemon-shaped, ends concolorous to paler, approaching hyaline, 7–12 × 6–8 μ.

Habitat: On dead leaves and culms of *Carex*, *Scirpus* and *Juncus* species.

Distribution: Portugal: Coimbra; Italy: Piedmont; France: Meaux; Germany: Dresden; Lithuania: Vidzeme; Switzerland: Neuchatel; England: Somersetshire.

Kunze, Myk. Hefte **2**: 105. 1823, listed *Sporophleum gramineum* as a synonym of this species but this was only a name Nees used in a manuscript according to D. P. Rogers.

4. ARTHRINIUM CURVATUM Kze. ex Fr., Syst. Myc. **3**: 377. 1832.
Arthrinium curvatum Kze., Myk. Hefte **2**: 103. 1823.
Camptoum curvatum (Kze.) Lk. in Willdenow, Linn., Sp. Pl. 4 ed. **6**(1): 44. 1824.
Menispora cinerea Corda, Icon. **1**: 16. 1837.

Spores falcate to rarely nearly straight, similar to *A. cuspidatum* but without the strong horn-like tips, brown, 9–12.5 × 5–7 μ.

Habitat: On dead leaves of *Scirpus*, *Carex* and *Juncus* species.

Distribution: North America: Pennsylvania: Clearfield; Europe: Germany, Austria, Italy, Latvia, Russia, Hungary.

Discussed more fully by Overholts in Mycologia **22**: 233. 1930.

Perfect state: *Pseudoguignardia scirpi* Gutner obtained in pure culture in Russia and in England.

5. ARTHRINIUM SPOROPHLEOIDES Fckl., Jahrb. Nass. Ver. Naturk. **27/28**: 78. 1873.

Spores reported as spindle-shaped, brown, 14 × 6 μ. The only specimen seen which was so labeled was *A. sporophlaeum*.

Habitat: On leaves of *Carex* spp.
Distribution: Europe: Switzerland.

6. ARTHRINIUM MORTHIERI Fckl., Jahrb. Nass. Ver. Naturk. **23/24**: 357. 1870.

Spores at first hyaline to pale, with a swollen basal area and a pointed tip, finally becoming brown, elongate-elliptic, 18–20 × 4–7 μ.
Habitat: On dead leaves of *Carex* spp.
Distribution: Europe: Switzerland.

7. ARTHRINIUM PUCCINIOIDES (DC.) Kze. ex Fr., Syst. Myc. **3**: 376. 1832.
Conoplea puccinioides DC., Flor. France **2**: 75. 1805.
Conoplea atra β caricarum Alb. & Schwein., Consp. 138. 1805.
Xyloma caricinum Fr., Obs. Myc. **2**: 361. 1818.
Arthrinium puccinioides (DC.) ex Kze., Myk. Hefte **2**: 103. 1823.
Goniosporium puccinioides (DC.) Lk. in Willdenow, Linn., Sp. Pl. 4 ed. **6**(1): 45. 1824.
Gonatosporium puccinioides Corda, Anleit. 61. 1842. (Error for *Goniosporium*)

Spores brown, rhomboidal rather than truly spherical, but nearly isodiametric, seated at one corner, 9–12 μ in diameter; conidiophore cells longer than in other species of the genus.
Habitat: On dead leaves of *Carex* sp.
Distribution: Europe: Germany.

8. ARTHRINIUM SPHAEROSPERMUM Fckl., Jahrb. Nass. Ver. Naturk. **27/28**: 79. 1873.
Goniosporium sphaerospermum (Fckl.) Sacc., Syll. Fung. **4**: 280. 1886.

No material of this species was available for examination, but from the description and treatment by Lindau it belongs in this group near the preceding species. The spores are reported as spherical to ellipsoid, brown and 7–8 μ in diameter or length.
Habitat: On dead leaves of *Phleum pratense*.
Distribution: Europe: Switzerland.

LIFE HISTORY AND RELATIONSHIP OF THE RUSTS OF SPARGANIUM AND ACORUS [1]

J. A. PARMELEE AND D. B. O. SAVILE [2]

(WITH 3 FIGURES)

Among well-known North American rusts whose aecial connections have not been demonstrated are *Uromyces sparganii* Clint. & Peck, on *Sparganium eurycarpum* Engelm., and *U. pyriformis* Cooke, on *Acorus calamus* L. These rusts have long been of additional interest because of their great morphological similarity. Peck (**13**) stated of *U. pyriformis*, "This species is very closely allied to *U. sparganii*, but appears to differ in habit." The meagre original descriptions of both species make it impossible to judge the basis for Peck's statement, but his examination of the specimens must have been more detailed than the descriptions suggest. P. and H. Sydow (**16**, p. 294) also noted the resemblance, saying, of *U. pyriformis*, "Mit *Urom. Sparganii* Cke. et Peck sehr nahe verwandt, aber anscheinend habituell durch die mehr verlängerten Sori verschieden." Their descriptions are very similar and the ranges of spore sizes are identical. Arthur, both in *North American Flora* (**1**, pp. 222, 237) and in his *Manual* (**2**, pp. 116, 215) gave descriptions that differed only in minor details, but did not suggest any relationship. It is possibly significant that Arthur followed essentially Engler's sequence for the Monocotyledoneae, wherein *Sparganium* and *Acorus* are widely separated; but the Sydows followed a sequence approximating that of Bentham and Hooker in which these genera are brought close together.

The similarity between these rusts was noticed some years ago by Mr. I. L. Conners, and both he and the junior author found that they were closely associated in the Ottawa district in 1946. It was felt that they might be a single species with a common aecial host, and a search for the aecial stage was planned. Unfortunately the Ottawa River remained in flood throughout May and June, 1947, owing to exceptionally heavy snow and rain. Not only were the only rust colonies then known in

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the district inaccessible, but all rusts in the river marshes were greatly suppressed, and those in question were extremely scarce in 1948. Field work in other parts of Canada prevented a renewed search for several years.

FIELD OBSERVATIONS

Both *Acorus* and *Sparganium* were freely rusted in a marshy inlet at Britannia, a suburb of Ottawa on the Ottawa River, in the fall of 1951. On 15 July 1952 a search of this area was made, bearing in mind that the aecial host was probably a plant known to harbour some other rust.

FIG. 1. Rusted *Acorus* and *Hypericum* in association near Ottawa, Ont. (about ½ natural size).

Hypericum virginicum L. was abundant at the edge of the marsh, growing, on the average, higher up the bank than *Acorus* and *Sparganium* but overlapping the zone of these plants considerably. Aecia were abundant on *H. virginicum* where it was closely associated with these plants, but not elsewhere. Later, uredinia on *Acorus* became abundant adjacent to the aecia (FIG. 1), and eventually spread elsewhere on both hosts. As soon as the associated aecia were seen it was recollected that in 1946 aecia had been collected on the same plant in the same area, but that no uredinia or telia had developed although these stages of *U. hyperici* had developed normally on *Hypericum ellipticum* Hook in

adjacent grassland. As the season advanced, rusted *Acorus* and occasionally *Sparganium* were found associated with *H. virginicum* at several locations near Ottawa. No infection could be found at any great distance from this plant until late in the season.

On 24 July a site near Bristol, Que., 35 miles west of Ottawa, where Mr. Conners had collected rusted *Sparganium* and *Acorus* in 1946, was visited. *Hypericum virginicum*, associated with *Acorus* near the high-water mark, bore abundant spent aecia; but plants in lower situations were uninfected, having evidently been submerged during basidiospore discharge. *Acorus* was heavily rusted near the infected *Hypericum*, but plants in wetter situations did not become rusted until late summer. The colony of *Sparganium curycarpum*, located by Mr. Conners in 1946, was not rediscovered. It may be noted, however, that *S. androcladum* (Engelm.) Morong, growing near the rusted *Acorus*, escaped infection.

INOCULATION EXPERIMENTS

The observed associations made it apparent that *Hypericum virginicum* was the aecial host for the rusts of both *Acorus* and *Sparganium*, and provided additional evidence that a single species was involved. Inoculation experiments were accordingly planned to prove the connection between the aecial stage and the rust on each telial host, and to see whether physiologically distinct races occurred on these hosts.

In late July 1952 *Hypericum* plants from Britannia, heavily infected with aecia, were transplanted to sites near the Dominion Arboretum and Botanic Garden, where *Acorus calamus* and *Sparganium curycarpum* were present and had been free of rust for several years. The lateness of the inoculation and shock of transplanting limited aeciospore discharge, and the primary infections were overlooked; but after four weeks uredinia were evident on each host in close association with the *Hypericum* plants. The rust eventually spread more than 50 yards. Some telia were present by 8 September.

A similar test was made in 1953 at a site a few miles south of Ottawa on the Rideau River, where *Acorus calamus* and *Sparganium curycarpum* grew singly or together in small clumps, but where *Hypericum virginicum* was absent. Infected plants of *H. virginicum* were placed in a mixed clump of *Acorus* and *Sparganium*. A month later both hosts in this clump were heavily rusted. Some infection occurred in adjoining clumps; but infection fell off rapidly with increasing distance from the aecial inoculum and none occurred more than 20 feet away.

In a greenhouse experiment, using potted plants, infection was ob-

tained on *Sparganium* but not on *Acorus*. Both controls remained healthy.

Some difficulty was experienced in establishing *Hypericum virginicum* away from the alternate hosts for use in reciprocal cultures. In the fall of 1952 six plots were established in a low area at the Dominion Arboretum and Botanic Garden, three in wet ground and three in moderately dry ground. One plot in each set was strewn with infected *Acorus* leaves, one with *Sparganium* leaves and one was used as a control. None of the *Hypericum* plants showed any growth up to early June 1953, and no germination of the teliospores had occurred. It appears that conditions at this site were not suited to the overwintering of either *Hypericum virginicum* or the teliospores. *Hypericum* plants in a very early stage of growth were accordingly secured and planted in these plots. By 10 July some aecia were present on the new transplants in the wet plot inoculated with telia on *Acorus* and one abortive infection occurred in the wet plot inoculated with telia on *Sparganium*. The *Sparganium* leaves were, as is usually the case, more lightly infected than those of *Acorus*, which, with low germination, rendered the inoculum from the former inadequate.

Although the original cultures proved that inoculum from *Hypericum virginicum* could infect both *Acorus* and *Sparganium*, there remained the possibility that the aecia were of two or more physiologically distinct races any one of which could infect only one telial host. This point has not been cleared up completely. There may be two or more races involved, but cross-inoculations have not been fully satisfactory. It is not easy to keep these marsh plants in vigorous condition in the greenhouse, away from risk of contamination, which may have been the cause of light infection or lack of infection in some tests. It has been our experience that, in mixed infections, *Acorus* is always more heavily infected than *Sparganium* in the Ottawa district. Both Peck (**12**, **13**) and the Sydows (**16**) noted that the sori on *Sparganium* are smaller than those on *Acrous*. It thus seems possible that *Sparganium* is uniformly the more resistant host, regardless of the possible occurrence of physiological races.

In the first uredinial transfer, spores from *Acorus* were spread on leaves of both hosts. Infection resulted on *Acorus* but not on *Sparganium*. This result might be due either to the inoculum being of a race specialized to *Acorus*, or to the apparent resistance of *Sparganium* to the species as a whole.

In a further test, heavily rusted plants of each species were planted in separate flats. One healthy plant of *Acorus* and one of *Sparganium*

were also placed in each flat. The flats were held for two days in moist chambers and then kept separately until the end of the experiment. The results are shown in TABLE I. One plant died, making it impossible to compare the rate of infection on the two hosts with spores from *Sparganium*. However, spores from *Acorus* gave infection on *Sparganium* and *vice versa*. The differences in intensity that were recorded could as well have been due to the apparent difference in susceptibility of the hosts to the rust as to any host specialization. It is significant that both hosts are commonly found rusted together, which, would not be expected if most races of the rust were restricted to one host. In addition to the associations recorded at Ottawa and Bristol, we have specimens on both hosts collected on the same date from L. Ainslie, N. S.; and Peck (**12**) reported both rusts from Montezuma marshes and New Baltimore, N. Y.

TABLE I

RECIPROCAL INOCULATIONS WITH UREDINIOSPORES BETWEEN ACORUS AND SPARGANIUM

Source of inoculum	Infection obtained on	
	Acorus	*Sparganium*
Acorus	Heavy	Light
Sparganium	Light	Plant died

Even if there is any degree of host specialization it must be minor, and, unaccompanied by any morphological difference, it provides no basis for varietal distinction.

GEOGRAPHIC RANGE OF RUST AND HOSTS

FIG. 2 shows the approximate distribution in North America of *Uromyces sparganii* and its aecial and telial hosts. *Sparganium eurycarpum* and *Acorus calamus* have nearly identical ranges, extending from Fla. to N. S., westward to central Alta., across the Rockies to southeastern B. C., southward east of the Cascades and Sierra Nevada, and eastward with somewhat different southern limits. The eastern part of the range of *Hypericum virginicum* is somewhat similar, but it is absent from the western half of the range of the telial hosts. It will be seen that all the published records of the rust on either telial host are within the range of *Hypericum virginicum*.

Uromyces pyriformis is recorded from Japan by Ito (**10**). Two specimens from Formosa and Okinawa (ex Herb. Hiratsuka) on *Acorus asiaticus* (*A. calamus s.l.*) are barely distinguishable from North American material. It is interesting to note that several species of *Hypericum*

sect. *Elodea* are recorded in the same general area by Gilg (**7**), viz. *H. fauriei, H. similans* and *H. virginicum* in Japan; and *H. taqueti* and *H. turfosum* in Korea.

Uredo acori Raciborski was described, on *Acorus terrestris* (*A. calamus s.l.*), from the Botanic Garden, Buitenzorg, Java. The type has not been seen and the original description is not very detailed, but it is treated by Arthur (**2**) and Ito (**10**) as synonymous with *Uromyces pyriformis*. The Sydows (**17**) noted its resemblance to *U. pyriformis*,

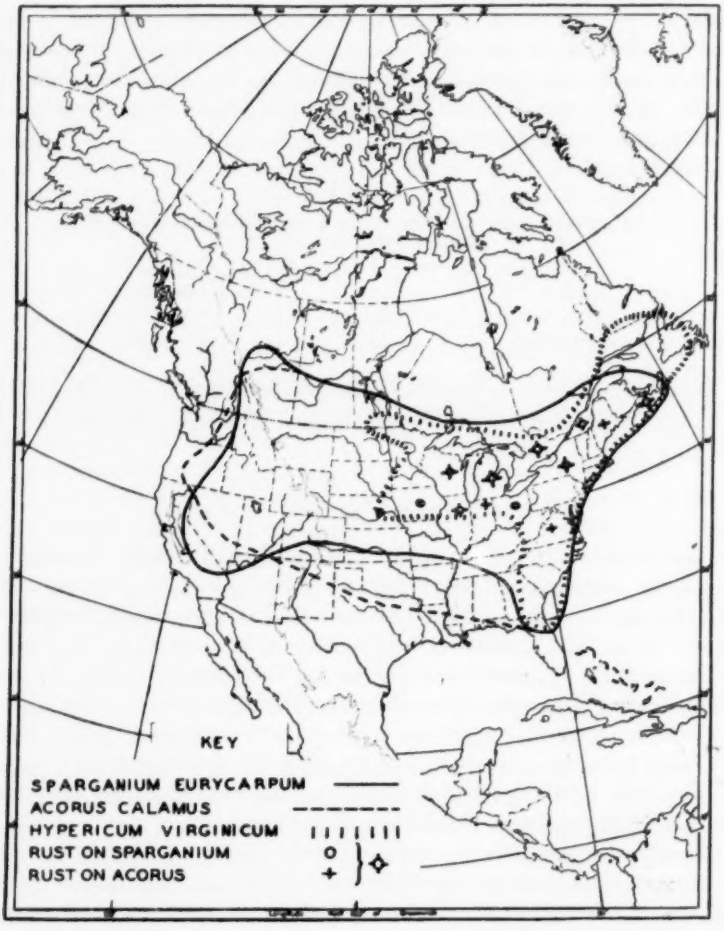

FIG. 2. Approximate ranges of *Uromyces sparganii* and its hosts in North America.

but had inadequate material for comparison. In Java the uredinia may be expected to persist throughout the year, eliminating any need for the alternate host.

Uredo acori was also reported by the Sydows (**17**) from India (Assam) and Borneo. The spores of Syd. Ured. *2143*, on *Acorus calamus*, from Gauhati, Assam, are very similar to those of *U. sparganii* on *Acorus* and *Sparganium* in North America, and identical with those of the specimens from eastern Asia cited above. It is uncertain whether the uredinia can persist at Gauhati (ca. 26° N), but Gauhati is in the Khasi district, whence Gilg (**10**) reports *Hypericum breviflorum* (sect. *Elodea*).

Recently Ramakrishnan and Rangaswami (**14**) described *Uromyces acori* on *Acorus calamus* from Ootacamund in southern India (ca. 11° 30′ N). The authors state that their species is identical with *Uredo acori*, but describe the presence of paraphyses in the uredinia, which should serve to separate it from *U. sparganii* (*pyriformis*). The writers have compared spore mounts and sections of *Uredo acori* (Syd. Ured. *2143*) and of the type collection of *Uromyces acori* with those of the North American rust on *Acorus calamus*. A detailed comparison is given in the next section, but it may be noted here that the differences seem to be small. The structures described by Ramakrishnan and Rangaswami as paraphyses do not resemble any uredinial paraphyses ever seen by us. They appear to be persistent uurediniospore pedicels made conspicuous by some accumulation of tannins or other substances. Possibly their persistence is genetically controlled, but it may well be an environmental effect. Fewer and usually paler residual pedicels are seen in Canadian specimens, but under our conditions a dense layer of teliospores is usually formed as soon as urediniospore formation slows down. Sori with sparse urediniospores and few or no teliospores have never been seen in our specimens.

MORPHOLOGY AND TAXONOMY

Although, as we have seen, the similarity between *Uromyces sparganii*, *Uromyces pyriformis* and *Uredo acori* has long been noted, the early descriptions were inadequate, particularly with regard to urediniospore morphology.

Uromyces sparganii was described by Clinton and Peck (**12**) as follows: "Sori minute, oblong, crowded, black, spores pyriform or oblong pyriform, about .001 in. long; pedicel colored, shorter than or equal to the spore in length. Both sides of leaves of *Sparganium*, Buffalo. Clinton." This species is widely credited to Cooke and Peck, but the

fungus was collected by Clinton and, as will be shown elsewhere, it has become apparent that Clinton was the co-author of many of the species attributed in Peck's reports to C. & P. or P. & C.

Cooke (**13**) described *Uromyces pyriformis* thus: "Amphigenous, erumpent; sori linear, sometimes confluent, rather pulverulent, purple-brown, margined by the fissured cuticle; pseudospores pyriform, deep-brown, epispore thickened above; pedicels rather short, thick, persistent, colored in the upper portion."

The Sydows (**16**) gave more detailed descriptions that differed somewhat for the sori, but were virtually identical for the spores. The urediniospores were described in each instance as globoid, subgloboid or ellipsoid, laxly echinulate, 20–28 × 18–25 μ, with wall ca. 1.5–2 μ thick; and the teliospores as ovate, pyriform or oblong clavate, rarely subglobose, the apex rounded, truncate or acutish, up to 12 μ thick, the base generally narrowed, smooth, 20–35 × 14–20 μ, with pedicel persistent, brownish. The urediniospore germ-pores were not mentioned.

Arthur (**1**) described *Uromyces sparganii* as having urediniospores 18–24 × 15–18 μ, wall 1–1.5 μ, rather finely and sparsely echinulate, the pores 2 or 3, equatorial; and teliospores 20–27 × 15–19 μ, wall 1.5–2 μ, 5–7 μ at apex, smooth. The urediniospores of *U. pyriformis* he described as being 21–26 × 18–19 μ, wall 1.5–2 μ, evenly and rather moderately echinulate, the pores usually 2, sometimes 3, approximately equatorial; and the teliospore 20–27 × 13–18 μ, wall 1.5–2 μ, 5–7 μ at apex, smooth. He later (**2**) gave almost identical descriptions of both rusts.

Uredo acori Rac. was described as having urediniospores globose or obovate, yellow or yellow-brown, echinulate, 24–28 × 22–24 μ. The Sydows (**17**) emended the description to read 23–28 × 19–23 μ, wall 2–2.5 μ thick, pores 2, equatorial. This description was presumably based in part on the Assam specimen (Syd. Ured. *2143*), which might conceivably be different from Raciborski's fungus; but the four Asiatic specimens available to us are all very similar and it is reasonable to suppose that Raciborski's material was identical with them.

Uromyces acori Ram. & Rang. (**14**) was described as having urediniospores 22–33 × 19.5–25 μ; and teliospores 18.6–36 × 16–25 μ, with the apex up to 14 μ thick. The sori were stated to have clavate paraphyses scattered through them. As already noted, these structures appear to be persistent urediniospore pedicels, somewhat colored by tannins or other substances and thus more conspicuous than usual. They do not constitute an important distinction from the North American rust.

A feature common to all the Asiatic and American specimens ex-

amined is that the urediniospores are somewhat flattened, have one pore situated on each flattened face, and have a circular area free from echinulations below or surrounding each pore (FIG. 3). The pores are equatorial to slightly superequatorial. These spores are intermediate between those of *Puccinia extensicola* and other species with strongly superequatorial pores subtended by the smooth area, and *P. helianthi* etc. with equatorial pores centered in the smooth area, two series of rusts discussed elsewhere (**15**).

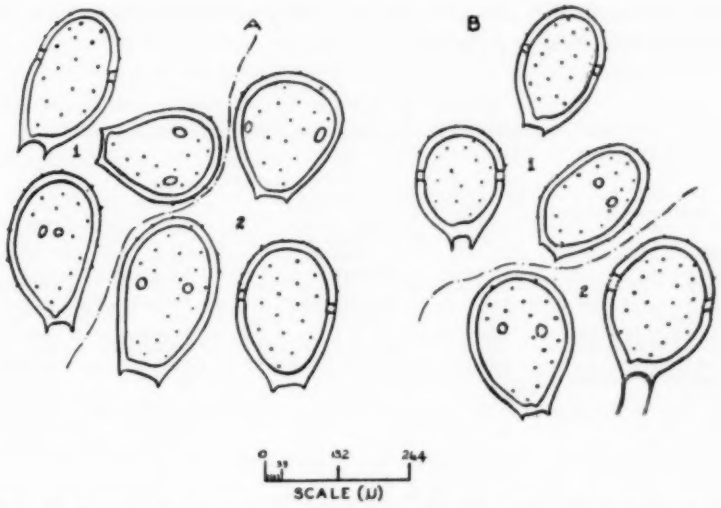

FIG. 3. Camera lucida drawings of urediniospores of *Uromyces sparganii*. A, from *Sparganium:* 1, F. Columb. *3896*, 2, DAOM *40576*. B, from *Acorus:* 1, DAOM *40590;* 2, *40591*.

Only a few teliospores are present in the available specimen of *Uromyces acori*, and there are none in the other Asiatic specimens examined. It is accordingly doubtful whether they differ appreciably from those of American specimens. TABLE II has been compiled to compare the urediniospores of the available Asiatic specimens with the range of a selection of North American specimens on *Acorus* and on *Sparganium*. The echinulation pattern is similar in all specimens and is deleted from the table to save space. Wall color is very similar in all specimens, but tends to be slightly deeper in the somewhat thick-walled Asiatic material than in the thinner-walled North American specimens. It will be seen that the four Asiatic specimens are very similar; but that they differ

from the North American collections in the spore length being slightly greater, the spore width being substantially greater, and the wall thickness being appreciably greater. The dimensions of the North American collections on *Acorus* and *Sparganium* agree very closely. From these figures it appears that the Asiatic and American populations are distinguishable, although each is essentially homogeneous. Since *Acorus calamus* is a common host the distinction appears to be due to geographic isolation, and the two populations should, therefore, properly be treated as subspecies.

To cope with this situation and to supply amplified descriptions the following treatment is proposed:

TABLE II

UREDINIOSPORE DIMENSIONS IN ASIATIC AND NORTH AMERICAN SPECIMENS

Specimens examined	Urediniospore characters			
	Length (μ)	Width (μ)	Wall (μ)	Number and position of pores
Type of *Uromyces acori*. S. India	23–28.5 (–33.6)	18.5–26.5	1.7–3.2	2, equat. –sl. super.
Uredo acori, Syd. Ured. *2143*. NE India	21.5–31.5	(17.2–)20–25.5 (–28.5)	1.6–2.8	2(–3), ±equat.
ex Hiratsuka *1455*, Formosa	23–31.5	18.5–26.5	1.8–3.0	2, equat. (–sl. super.)
ex Hiratsuka *1456*. Okinawa I.	23–34	18.5–28.5	1.6–3.2	2, equat. (–sl. super.)
Range of 5 scattered North American collections on *Acorus*	18–28.5	(13.5–)15.5–21.5	1.1–2.1	2(–3), equat. –sl. super.
Range of 5 scattered North American collections on *Sparganium*	18.5–28.5 (–31.5)	14.5–21	1.0–1.9	2(–3), equat. –sl. super.

UROMYCES SPARGANII Clint. & Peck apud Peck, Ann. Rep. N. Y. State Mus. **26**: 77. 1874, ssp. SPARGANII.

Uromyces pyriformis Cooke apud Peck, Ann. Rep. N. Y. State Mus. **29**: 69. 1878.

Nigredo sparganii Arth. N. Am. Flora **7**: 222. 1912.

Nigredo pyriformis Arth. N. Am. Flora **7**: 237. 1912.

Pycnia amphigenous or caulicolous. Aecia hypophyllous or caulicolous, rarely epiphyllous, cupulate. Peridium lacerate, white. Peridial cells closely and distinctly verrucose on inner face, abutted, generally hexagonal in surface view. Aeciospores 16–22 × 13.5–18.5 μ, subglobose to oblong-ellipsoid; wall 0.5–1.2 μ thick, hyaline, usually patchily verrucose with minute to moderately large warts.

On *Hypericum virginicum*, probably nearly throughout range, but confused with similar aecial stage of *Uromyces hyperici* on this host.

SPECIMENS EXAMINED: *Hypericum virginicum* L., Ont. (Ottawa

and vicinity, DAOM *19198, 40582, 40583, 40596, 40597*); Que. (Bristol, Pontiac Co., DAOM *40594;* East Templeton, Papineau Co., *40595;* Rivière Ouelle, Kamouraska Co., *20834*).

Uredinia amphigenous, cinnamon-brown, pulverulent. Urediniospores subglobose, broadly ellipsoid or obovoid, usually slightly flattened on areas bearing pores, 18–28.5(–31.5) × (13.5–)14.5–21.5 μ; wall 1.0–2.1 μ, slightly thicker at pores, yellow-brown, sparsely but distinctly echinulate except for a smooth circular area ca. 10–15 μ diam. centered about or below each pore; germ-pores 2(–3) equatorial to slightly superequatorial. Telia amphigenous, blackish brown. Teliospores pyriform, obovoid or subglobose, 17.5–34.5(–38.5) × (11.5–20 (–23) μ; wall 1.2–2.0 μ at sides, 3.0–8.5(–9.5) μ above, chestnut brown, smooth; pedicel persistent, brownish, about length of spore.

On *Sparganium curycarpum* and *Acorus calamus*, eastern North America.

SPECIMENS EXAMINED: *Sparganium curycarpum* Engelm., Ont. (Ottawa district, DAOM *40576, 40577, 40578, 40579, 40580, 40581, 40598;* SE. L. Huron, F. Columb. *3893*, N. Am. Ured. *998*); Que. (Bristol, Pontiac Co., DAOM *19037;* Rivière Ouelle, Kamouraska Co., *20835*); N.S. (L. Ainslie, DAOM *5167*); Neb. (F. Colomb. *2398*, N. Am. Ured. *197*); Ohio (Kell. Ohio F. *200*). *Acorus calamus* L., Ont. (Ottawa district, DAOM *19201, 19203, 40587, 40589, 40590, 40591, 40592, 40593*); Que. (Bristol, Pontiac Co., *19028, 19202, 40585;* East Templeton, Papineau Co., *40586, 40588;* Waterloo, Shefford Co., *19238*); N.S. (L. Ainslie, DAOM *5218*); Iowa (N. Am. Ured. *699*); Wis. (F. Columb. *3098*); Mich. (F. Columb. *4096*, N. Am. Ured. *598*); Ind. (U.S.D.A. Myc. Exch. of 1921 *885*); N.Y. (N. Am. Ured. *2698*).

UROMYCES SPARGANII Clint & Peck ssp. **asiaticus** nom. nov.

Uromyces acori Ram. & Rang. Current Sci. **17**: 240–241. 1948.

? *Uredo acori* Rac. Parasit. Algen und Pilze Javas I, 28. 1900.

Pycnia and aecia unknown, probably on *Hypericum* sect. *Elodea*.

Differs from ssp. *sparganii* in its larger urediniospores, 21.5–31.5 (–34) × (17)–18.5–26.5(–28.5) μ, with walls thicker, 1.6–3.2 μ. Urediniospore pedicels tend to be more persistent in Indian specimens seen than in ssp. *sparganii*, but not noticeably so in specimens from eastern Asia; tendency may be climatic rather than genetic.

On *Acorus calamus s.l.*, southern and eastern Asia.

SPECIMENS EXAMINED: *Acorus calamus* L. vars., southern India (Ramakrishnan and Rangaswami, as *Uromyces acori*, type); Assam

(Syd. Ured. *2143*); Formosa (DAOM *33136*, ex Hiratsuka *1455*); Loochoo Is. (Okinawa, DAOM *33137*, ex Hiratsuka *1456*).

The range of ssp. *asiaticus* is not known in detail. It follows *Acorus calamus* into the tropics, where it survives in the uredinial state. Farther north its distribution may prove to be largely that of the aecial hosts, which would account for it not having followed *Acorus calamus* into Europe. The markedly disjunct distribution pattern of *Hypericum* sect. *Elodea* is one that Dr. B. Boivin points out to be characteristic of various tertiary genera, e.g. *Magnolia, Liriodendron, Podophyllum* and *Menispermum*. As Cain (**5**, p. 253) indicates, this pattern was first reported by Asa Gray nearly a century ago. The two populations of the rust have, therefore, evidently been separated at least since late tertiary times, which makes it apparent that *Uromyces sparganii* is a decidedly conservative species.

There are presumably numbers of specimens of the aecial stage of *Uromyces sparganii* filed in herbaria under *U. hyperici*. Unfortunately *Hypericum virginicum* also takes the latter rust and the aecia do not seem to be safely distinguishable. In fact there seems to be more variation in *U. hyperici* on various hosts than between the two rusts on *H. virginicum*.

RELATIONSHIP OF ACORUS AND SPARGANIUM

Engler's system of classification of the flowering plants, which assumed world-wide popularity with the publication of *Die Natürlichen Pflanzenfamilien*, is still used with only minor modifications by many authors, e.g. Fernald (**6**) and Lawrence (**11**), despite its admitted faults. Its popularity is due in part to its completeness in contrast with some other suggested arrangements. To anyone accustomed only to the Engler system the statement that a single rust will infect *Sparganium* and *Acorus*, in the supposedly widely separated orders Pandanales and Spathiflorae, respectively must seem strange; but Bentham and Hooker (**3**), Bessey (**4**) and Hutchinson (**8**, **9**) all place Sparganiaceae and Araceae much closer together. In its modified form Hutchinson's (**9**) arrangement of the Monocotyledoneae, which is partly an outgrowth of Bessey's proposals, seems to be the most satisfactory treatment published to date. In it we find Arales and Typhales (including Sparganiaceae) brought closer together, with the suggestion that each arose more or less independently from Liliaceae, through Aspidistreae. The present findings suggest that the affinity may be even closer than Hutchinson supposed. In view of the biochemical relationship indicated by susceptibility to the same rust, it seems probable that the other resemblances between *Sparganium eurycarpum* and *Acorus calamus* may be due to

relationship rather than to convergent evolution. The habitat and habit of these plants are very similar. In the vegetative state care must be taken to distinguish them, although with practice the keeled leaf of *Sparganium* is readily told from the symmetrical leaf of *Acorus* by pressing it between finger and thumb. It must also be noted that, although the inflorescences are quite different in appearance, the individual fruits are remarkably similar. *S. eurycarpum* is distigmatic and is thus to be considered more primitive than the remaining, monostigmatic species. If we agree with Hutchinson that *Acorus* is a primitive aroid, i.e. that the ensiform bract is a primitive spathe rather than a reduced one, then *A. calamus* may be considered the most primitive member of Arales and *S. eurycarpum* the most primitive of Typhales; and it is not difficult to believe that each may have arisen more or less directly from a common ancestor.

SUMMARY

Uromyces sparganii, on *Sparganium eurycarpum*, and *U. pyriformis*, on *Acorus calamus*, are shown to be morphologically identical and cross-inoculable, and to have their aecial stage on *Hypericum virginicum*. They are accordingly combined under *U. sparganii*. The rust in Asia, which has been called *Uredo acori* and *Uromyces acori*, has slightly larger urediniospores and is treated as *U. sparganii* ssp. *asiaticus*. The range of the rust in eastern North America and eastern and southern Asia is evidently limited, except in the tropics, by the range of *Hypericum* sect. *Elodea*. It is suggested that *Acorus* and *Sparganium* are more closely related than has been recognized.

ACKNOWLEDGMENTS

Thanks are due to Mr. A. Payette for specimens from the lower St. Lawrence, and to Mr. T. S. Ramakrishnan for type material of *Uromyces acori*.

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NOTES AND BRIEF ARTICLES

Discomyceteae exsiccatae, Fasc. I

A new exsiccatum devoted to discomycetes is being prepared under the editorship of the author and published by the Department of Plant Pathology, Cornell University. The first fascicle of twenty-five numbers will be issued November first, 1954. Eleven sets are being prepared for deposit in the following herbaria:

BPI = National Fungus Collections, Beltsville.
CUP = Dept. Plant Pathology, Cornell University, Ithaca.
DAOM = Mycological Herbarium, Dept. Agriculture, Ottawa.
K = Herbarium, Royal Botanic Gardens, Kew.
LAH = Dept. Botany, University of Panjab, Lahore.
MICH = University Herbarium, University of Michigan, Ann Arbor.
NY = Herbarium, New York Botanical Garden, New York.
PC = Lab. de Cryptogamie, Muséum National d'Histoire Naturelle, Paris.
PR = Botanical Department, National Museum, Prague.
UC = Herbarium, University of California, Berkeley.
UPS = Institute of Systematic Botany, University of Uppsala.

Collections of discomycetes suitable for division into eleven ample parts will be gratefully received, whether they have been determined or not. The first fascicle consists of fifteen operculate and ten inoperculate species, as follows:

1. Caloscypha fulgens (Pers.) Boud.

This is certainly unrelated to *Pseudoplectania*, where Seaver (**7**) ranges it.

2. Discina leucoxantha Bres.

The hymenium is often almost as dark as in the next species, though occasionally distinctly yellow.

3. Discina perlata (Fr.) Fr.

Seaver (**7**) terms this *D. ancilis*, but see No. 6 below. I am following Boudier's (**1**) interpretation.

4. DISCIOTIS VENOSA (Pers. *ex* Pers.) Boud.

A very characteristic species; it is in reality a cupulate *Morchella* on spore and ascus characters, and has no close relationship with *Peziza*, where it is placed by Seaver (**7**).

5. GYROMITRA ESCULENTA (Pers. *ex* Pers.) Fr.

This is the characteristic spring form of the U. S. The guttules in the spores are larger than Boudier (**1**) draws for this species, and approach those of *G. infula*. Seaver (**7**) treats this as *Helvella infula* pro parte.

6. HELVELLA ANCILIS (Pers. *ex* Pers.) Quél.

Persoon's species appears to be this, at least in the sense of Boudier (**1**), who terms it an *Acetabula*. I prefer to follow Nannfeldt (**5**) and merge these genera. In Seaver (**7**) it would perhaps be *Paxina acetabulum* pro parte.

7. OCTOSPORA LEUCOLOMA Hedw. *ex* Gray

I designate this species as the LECTOTYPE of *Octospora* Hedw. *ex* Gray, Nat. Arr. Br. Pl. **1**: 666. 1821. [*O. leucoloma*, p. 667.] This gives us a valid generic name for many of the *Humaria-Humarina* species. *Humarina* Seaver, an obligate synonym, is nomenclaturally superfluous, as it included the holotype of an earlier, valid generic name (*Leucopezis* Clements), and therefore cannot be used if we follow the International Code.

8. **Octospora tetraspora** (Fckl.) Korf, comb. nov. (Basionym: *Ascobolus tetrasporus* Fckl. *in* Hedwigia **5**: 4. 1866.)

The new combination, though necessary, is unfortunate!

9. **Peziza badio-confusa** Korf, nom. nov. (Basionym: *Aleuria olivacea* Boud. *in* Bull. Soc. Myc. Fr. **13**: 14. 1897, non *Peziza olivacea* Batsch *ex* Pers. 1822.)

This species is almost invariably determined in America as *Peziza badia*, and certainly is that figured by Seaver (**7**: pl. 54, *1*) under this name. It is perhaps our commonest large cup-fungus, and it seems to me that it must have been described before 1897 by some European or American author, but I have seen no types which match it. The true

P. badia has reticulate spore-markings, as shown by Le Gal (**4**). (The only American material I have seen which might be the true *P. badia* is from Wisconsin and from the Rocky Mountains.) As LECTOTYPE of the genus *Peziza* Hooker, Fl. Scot. **2**: 32. 1821, I designate *P. vesiculosa* Sow. *ex* Hook. (*loc. cit.*, p. 33).

10. PEZIZA GERARDII Cke.

This is a not uncommon species, very easily characterized by its spores, and a true *Peziza* (*Galactinia*), classed by Seaver (**7**) in *Humarina* because of its small size. The asci turn blue in iodine, as is characteristic of the genus. Le Gal (**4**) terms it *Galactinia ionella*, a later synonym.

11. PEZIZA HOWSEI Boud.

This remarkable species is apparently rare and heretofore unreported with us. It matches Boudier's plate (**1**) of *Aleuria lilacina* Boud. almost exactly in color and in outward appearance, though the spores are very different and correspond to the type shown by Le Gal (**4**) for *P. howsei*.

12. RHIZINA UNDULATA Fr. *ex* Pers.

13. SARCOSCYPHA COCCINEA (Jacq. *ex* Pers.) Lambotte var. COCCINEA

This is Rosinski's (**6**) tapered-spore type.

14. SARCOSCYPHA OCCIDENTALIS (Schw.) Sacc.

Seaver's (**7**) generic name is *Plectania* for this and the preceding, but see Korf (**3**).

15. TRICHOPHAEA ABUNDANS (Karst.) Boud.

One of the *Lachnea* generic complex, this species is treated under *Patella* in Seaver (**7**).

16. ANGELINA RUFESCENS (Schw.) Duby

17. ARACHNOPEZIZA AURATA Fckl.

18. ARACHNOPEZIZA CORNUTA (Ell.) Korf

Fresh material of this species has shown me what I missed in dried specimens. The spore appendages described by Ellis which I consid-

ered undemonstrable can be made out in living, unruptured asci. The epiplasm appears to cleave into tapering portions at each end of the spore. This "appendage" is not visible after the spore is shot, nor in dried material, as I reported in my monograph (2).

19. BULGARIA INQUINANS (Pers. *ex* Hook.) Fr.

The only operculate species of the original ones in *Bulgaria* Fr. was *B. globosa*, which was removed as the holotype of *Sarcosoma*. *Bulgaria* must thus be used for the inoperculate species. *Phaeobulgaria* Seaver falls in synonymy.

20. DASYSCYPHUS CORTICALIS (Pers. *ex* Mérat) Mass.

Dasyscyphus Gray dates from 1821 (Nat. Arr. Br. Pl. **1**: 670), and includes many species later placed in *Dasyscypha* Fckl. (1870) and *Lachnum* Retz. *ex* Karst. (1871). *Dasyscypha* Fuckel should be considered merely an orthographic variant, and combinations using either spelling should be considered valid, thus avoiding wholesale name changes. I designate *D. virgineus* (Pers. *ex* Hook.) Gray (*loc. cit.*, p. 671) as the LECTOTYPE of the genus.

21. DASYSCYPHUS NUDIPES (Fckl.) Sacc.

This species is scarcely distinct from the ubiquitous *D. virgineus*.

22. HYALINIA CRYSTALLINA (Quél.) Boud.

Rarely reported, but I have found this to be not uncommon in small numbers on very decayed wood.

23. MICROGLOSSUM RUFUM (Schw.) Underw.

24. PESTALOPEZIA RHODODENDRI Seaver

25. TRICHOGLOSSUM FARLOWII (Cke.) Durand

—RICHARD P. KORF, Dept. Plant Pathology, Cornell University.

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NOMINA GENERICA CONSERVANDA FOR FUNGI

The generic names approved for conservation by the Special Committee for Fungi and so reported in Mycologia **45**: 312–322, 476. 1953, and in Taxon **2**: 29–32, 102. 1953, were accepted by the Eighth International Botanical Congress at Paris. The cited pages in Mycologia carry the complete list of n.g.c. for Fungi; the enumeration in Taxon is supplementary to the one in Int. Code Bot. Nomencl. 76–78. 1952. Other proposals are before the Committee; no others have been approved.—DONALD P. ROGERS, Secretary, Special Committee for Fungi, The New York Botanical Garden, Bronx Park, New York 58, N. Y.

THE NATIONAL FUNGUS COLLECTIONS

As part of the reorganization of the U. S. Department of Agriculture which has been under way for some months, the unit heretofore known as the Mycological Collections of the former Bureau of Plant Industry, Soils and Agricultural Engineering, has been designated as The National Fungus Collections. This unit will operate as a cooperative project between the Horticultural Crops Research Branch of the Department of Agriculture and The Smithsonian Institution for the maintenance and upbuilding of the mycological herbaria of both institutions. These collections, with supporting files, catalogues, and the mycological library, will continue to be maintained at the Plant Industry Station, Beltsville, Maryland.—JOHN A. STEVENSON, Curator.

APPLICATIONS FOR GRANTS IN 1955

A portion of the annual income received from Sustaining Memberships is set aside for grants-in-aid to regular members of the Mycological Society of America. These grants are awarded to "promote interest" and "to further research" in the field of mycology. A grant ordinarily does not exceed $100 and not more than three grants are made to any one applicant in a five-year period. In rare instances a larger grant may be awarded but is then not renewable. Application blanks may be obtained from Dr. E. S. Beneke, Department of Botany, Michigan State College, East Lansing, Michigan. Applications, submitted in triplicate, must be in the hands of the Chairman of the Research Grants Committee by February 1, 1955.

Applications for grants-in-aid are not evaluated on any set of rigidly fixed standards, and the Committee will welcome a wide variety of requests. The funds available are not large, however, and it seems appropriate at this time to indicate the general philosophy which has been developed as a guide to making awards. The Committee feels that small grants will promote interest and research in mycology most effectively if they are awarded for the most part where the needs are most acute. Special consideration will be given, therefore, to those applicants who have least access to other research funds: to younger mycologists in the early years of their careers, to investigators in smaller institutions where internal funds are particularly limited, and to those who are engaged upon projects which do not ordinarily derive ready support from large granting agencies such as the Institutes and Foundations. Younger applicants are urged to provide supporting recommendation from some senior research investigator with whom they have been associated. Applications for funds to defray expenses of collecting trips will be considered only when they reveal urgent need closely associated with a sound research program.

For the Committee on Research Grants:

JOHN EHRLICH (Chairman of the Committee)—Research Laboratories, Parke, Davis & Co., Detroit 32, Michigan.

MYCOLOGICAL SOCIETY OF AMERICA

The Mycological Society will participate in the A.A.A.S. meeting at Berkeley, California, December 26–31, 1954. The following program has been arranged:

Tuesday afternoon, Dec. 28. Symposium: Physico-chemical control of structural differentiation in the fungi. Speakers, Robert M. Page, Edward C. Cantino, Kenneth B. Raper, Jackson W. Foster.

Tuesday evening, Dec. 28. Phytopathologists' banquet; A.A.A.S. Presidential Address; Reception.

Wednesday morning, Dec. 29. Demonstration of methods of assay for soil-inhabiting microorganisms; Discussion on soil-borne virus diseases of plants.

Wednesday afternoon, Dec. 28. Symposium: The mode of survival of plant pathogens in the soil.

REPORT OF THE MANAGING EDITOR OF MYCOLOGIA

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED JUNE 30, 1954

Cash receipts		
Joint funds		
Members' subscriptions (Mycological Society)	$ 3,173.00	
Other subscriptions	5,780.53	
Sales of V. 25 and later	1,366.63	
Excess pages and illustrations	353.39	
Reprints	.75	$10,674.30
Special funds		
Sales of V. 1–24 and Index	730.91	
Income from endowment	725.00	1,455.91
Total receipts		$12,130.21
Cash disbursements		
Printing and distribution	10,386.02	
Reprinting	673.62	
Office expense	399.38	
Total expenditures		11,459.02
Excess of receipts over expenditures		$ 671.19
Unexpended balance July 1, 1953		3,624.78
Unexpended balance July 1, 1954		$ 4,295.97
Endowment		$14,000.00

The above MYCOLOGIA funds are administered by The New York Botanical Garden, and the balances at June 30, 1954, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Company.

DONALD P. ROGERS, *Managing Editor*

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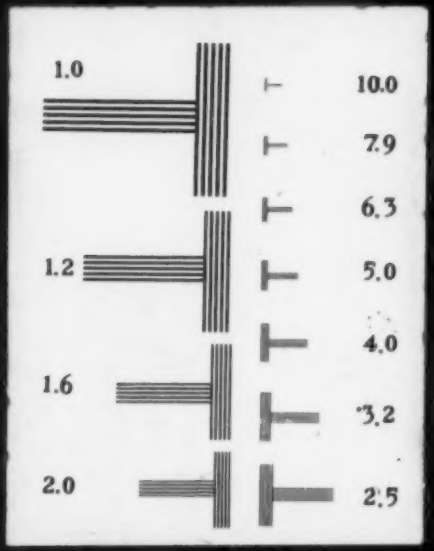

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